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AN INTRODUCTION TO BOTANY

AN INTRODUCTION TO BOTANY

WITH SPECIAL REFERENCE TO THE
STRUCTURE OF THE FLOWERING PLANT

BY

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PREFACE TO THE SECOND EDITION.

THE general arrangement and content of the book have not been altered appreciably, but in numerous minor points the subject matter has been corrected and brought up to date. A section has been added to the chapter on Respiration introducing recent advances in knowledge of oxidase systems and some modification has been introduced with regard to transpiration, water absorption and the conception of auxins. I am particularly grateful to Dr. R. D. Preston for advice in revision of the physiological sections and also to Professor M. Skene for comments.

The question of vascular nomenclature is a difficult problem. The usage of terms in this connection in the first edition of the book has been criticised by some and appreciated by others. It has, however, been felt, with some regret, that it is probably wiser to accept the more classical usage of the terms, so far as it has proved possible to determine their exact meaning. I am grateful to Professor W. H. Lang, Mr. C. R. Metcalfe and Professor D. Thoday for helpful criticism in connection with the anatomical sections.

The Latin names have been modified in the text or in Footnotes to follow the Check List of British Vascular Plants published by the British Ecological Society in the *Journal of Ecology*, Vol. 33, 1946, except that capital

initial letters have been retained for certain specific names in accordance with the present Rules of Nomenclature.

The senior author, Professor Joseph Hubert Priestley, died on the 31st of October 1944. A number of the modifications are the result of discussion between us during his lifetime, but it has been a great sorrow to me that I have not been able to draw upon his wisdom and wide knowledge and experience during the final preparation of this Edition.

1949

LORNA I. SCOTT

PREFACE.

THE material presented in these pages has, in the main, formed the basis of the first year's course in Botany in the University of Leeds. It is hoped that its appearance in print may give the lecturer greater freedom to digress and the student to listen. As teachers and students elsewhere may also make use of these pages, a statement follows of certain considerations which have influenced the selection of the subject-matter.

Topics so throng upon the lecturer in an introductory course in Botany that the chief difficulty is always to select, and in every University this problem may have been solved upon different lines. In Leeds, as the title of this book indicates, attention has mainly been directed to the flowering plant; this has been the natural outcome of the fact that much time and energy must be spent in learning the use of a new tool, the microscope. Preliminary training in observation and description precede microscopic work, specimens being handled, dissected, and sketched for the first few weeks, commencing with the seed, progressing to seedling and shoot and culminating with the dissection under the lens of a series of buds; the microscope is then brought in to enable the examination of the bud to be carried further.

The new tool is thus introduced to investigate the growing point, a problem which stresses the significance of development in the interpretation of form and structure. At an earlier stage function and form have

been linked by associating adult foliage with the most important physiological attribute of the plant, the gain in dry weight, but in these pages it is development rather than function and purpose that will be utilised in the interpretation of form and structure.

With the help of the microscope the student is introduced to the cell, and the organisation of the higher plant is approached through a brief review of unicellular and more simply organised plants. Such types are not considered again until the discussion of the essential organs of the flower necessitates a review of their life cycles to throw light upon the complex procession of events in sexual fertilisation and seed production. Most of the students taking this course proceed later to a systematic examination of the classes of the vegetable kingdom but, while the main class studies parasitism and saprophytism in the flowering plants, other students are introduced to parasitic rusts and saprophytic moulds and the significance of processes of sterilisation and methods of pure culture.

Plant physiological experiments in two-hour laboratory periods must necessarily be restricted in scope and an effort has simply been made, in considering metabolism and water relations, to show how physical and chemical principles lie at the basis of an understanding of the functional working of the plant.

In the study of the structure of the higher plant, methods have been particularly designed to make the student visualise the images seen in the field of the microscope as three dimensional objects, built up of substances which account for the behaviour of the living organism. This characteristic of the course at Leeds dates back before the present writers; the method of study of leaf structure was developed by Mr. Norman Walker under the direction of the late Professor Miall.

As this method, as well as the wide use made of chromic acid as a maceration agent, and the use of strip preparations for the study of the tissues derived from the cambium, have evolved in the Department, sufficient details are given of the technique to permit of their trial elsewhere. The photographs supply some evidence of their possibilities, as does also the fact that since the introduction of the maceration method for phloem, it has been possible to guarantee that every student will see sieve tube elements in his own material, and that probably 50 per cent. will find them without assistance.

As the majority of the students in the class will become teachers, details of simple demonstrations, suitable for school work, are also included. As examples may be cited the simple experiments on germination and water absorption by seeds, rubbings as records of branch scars, descaling experiments with buds, plasticine models of growing point cells. The same considerations determined the inclusion of numerical results relating to the entry of water by the micropyle of the seed, and the loss of water from the vased leaf.

It is at the same time the delight and despair of the teacher that the most elementary account of the working, and even of the structure of the plant, must continually venture outside the realms of ascertained fact. No connected story can be written of the plant without connecting-links woven of speculative material, many links indeed are not yet forged, they are still plastic in the fires of controversy. Where so little is sure, all generalisations remain suspect, and particular care has therefore been taken to describe and discuss facts with reference to named plants; the descriptions should thus stand, whatever may be the fate of the generalisations.

It has been a great encouragement to the writers of this book that Miss Malins' services have been available

for its illustration. Many times in the course of the work a written description has needed amendment on account of new information supplied by the accuracy of the drawing. Miss Malins' name appears on the title-page in recognition of the fact that, thanks to her whole-hearted collaboration, her illustrations are an integral part of the book.

It has been necessary to enlist help with some of the illustrations, and in this connection we have to acknowledge particularly the assistance given by Miss K. M. Mattinson. Photographs prepared by the University photographer, Mr. Manby, have been freely drawn upon; we are also indebted to other sources for the following illustrations: Figs. 133 and 134 of *Polygonum divaricatum* by Strasburger; Fig. 140a of conjugation in *Spirogyra*, which is adapted from photographs by Professor F. E. Lloyd; and Fig. 120d of the germinating teleutospore of *Puccinia graminis* by Tulasne. Acknowledgment is also due to the Council of the Leeds Literary and Philosophical Society for their kindness in lending a number of blocks.

CONTENTS.

CHAPTER	PAGE
I. THE SCOPE OF BOTANY. THE DIFFERENT KINDS OF PLANTS	1
II. THE SEED AND GERMINATION	7
III. THE SEED AND GERMINATION— <i>contd.</i>	28
IV. THE CONTINUED GROWTH OF THE SEEDLING AND THE ASSO- CIATED GAIN IN DRY WEIGHT	49
V. THE ADULT VEGETATIVE SHOOT	58
VI. THE LIFE CYCLE OF THE INDIVIDUAL PLANT	68
VII. HERBACEOUS AND WOODY PERENNIALS	76
VIII. HERBACEOUS POLYCARPIC PERENNIALS. MONOCOTYLEDONS	89
IX. THE WOODY PERENNIAL. I.	114
X. THE WOODY PERENNIAL. II.	129
XI. THE BUD SCALE. AN INTRODUCTION TO THE PROBLEM OF LEAF SHAPE	142
XII. THE INTERPRETATION OF LEAF SHAPE. THE GENERAL PROBLEM	154
XIII. THE ARRANGEMENT OF LEAVES ON THE STEM	168
XIV. AN INTRODUCTION TO THE MICROSCOPE	174
XV. THE SHOOT APEX	195
XVI. THE STRUCTURE OF THE LEAF	211
XVII. METABOLISM. THE LEAF AS A CHEMICAL LABORATORY	225
XVIII. THE CARBON ATOM. CARBOHYDRATES, FATS, PROTEINS	232
XIX. PHOTOSYNTHESIS	258
XX. RESPIRATION	266
XXI. EVAPORATION FROM THE LEAF	280

CHAPTER	PAGE
XXII. STEM STRUCTURE IN THE HERBACEOUS DICOTYLEDON. I.	292
XXIII. STEM STRUCTURE IN THE HERBACEOUS DICOTYLEDON. II.	313
XXIV. THE WOODY STEM. HARDWOOD AND SOFTWOOD . . .	327
XXV. THE RADIAL GROWTH OF THE TREE	348
XXVI. THE STRUCTURE OF THE MONOCOTYLEDON SHOOT . . .	367
XXVII. GROWTH CURVATURES. THE ROOT SYSTEM	379
XXVIII. THE STRUCTURE OF THE ROOT	394
XXIX. ENTRY OF WATER INTO THE ROOT. THE WATER BALANCE IN THE PLANT	410
XXX. THE ENTRY OF INORGANIC SOLUTES. THEIR RÔLE AND THEIR SUPPLY TO THE PLANT	419
XXXI. SPECIAL NUTRITION IN FLOWERING PLANTS	432
XXXII. FUNGI AS PARASITES AND SAPROPHYTES. STERILISATION AND THE TECHNIQUE OF PURE CULTURE	448
XXXIII. THE REPRODUCTIVE PHASE. THE FLOWER. THE STAMEN	468
XXXIV. THE CARPEL AND FERTILISATION	482
XXXV. LIFE CYCLES THROUGHOUT THE PLANT KINGDOM. I. NON- VASCULAR PLANTS. ALGÆ AND MOSSES	500
XXXVI. LIFE CYCLES. II. VASCULAR PLANTS. FERNS, GYMNO- PERMS AND ANGIOSPERMS	520
XXXVII. THE NUCLEUS AND HEREDITY	539
XXXVIII. BIOLOGY OF FLOWER AND FRUIT	556
XXXIX. PLANT CLASSIFICATION	594
INDEX	609

CHAPTER I.

THE SCOPE OF BOTANY. THE DIFFERENT KINDS OF PLANTS. .

The Beginnings of Botany.

From very early times, plants must have been of direct interest to man, for the simple reason that they form the ultimate source of his food supply. Almost alone, amongst living organisms, green plants are able to build up from the inorganic materials found in soil, air, and water, those complex organic substances, such as sugars, starches, and proteins, which are essential constituents of human and animal food. Very early in the history of the human race, therefore, certain kinds of plants were selected and cultivated as food for man or beast, but even up to the present time, relatively few kinds of plants are grown as crops, and it is probably to the use of herbs in primitive medicine that we owe the impetus to a more extended study of plants. Very appropriately, therefore, the word Botany is derived from the Greek word for a herb.

When the early herbalist found a plant to be effective in the treatment of some complaint, he had a strong incentive to make a faithful note of the appearance of the plant, so that, not only might he recognise it again himself when occasion arose, but also pass on his experience. Such recognition and description of a plant requires the closest observation and the most scrupulous care in the use of language. If the beginner has any doubt of this statement, let him try, before he has proceeded further with the study of botany, to describe, without the aid of sketches, any familiar plant so that a friend may recognise it from his description. He will then realise the difficulties of the task, and will probably become convinced of the necessity for formal categories of the parts of plants and the standardised use of descriptive terms, in fact for the

generally adopted terminology in use by the "taxonomic" botanist, whose task it is to describe plants. As he struggles to reduce to concise phraseology the variety of form shown by any common type of plant, there will arise, even in the mind of the least sympathetic observer, some degree of interest and speculation as to the causes governing the production of these vegetable structures, as to their construction and the processes which go on within them. It is the inevitable presentation of such queries to the human mind, as plants are handled and described, that leads to the science of botany, which represents an attempt to put these problems into words and to offer explanations of them.

The Classification of Plants.

When a number of plants are carefully studied and described, it immediately becomes apparent that there are degrees of likeness among them. The recognition of this fact leads naturally to an attempt to group the names of plants so that those most alike should be found close to one another. Such an arrangement would be valuable, because it would facilitate the process of finding the name of the plant on later occasions, but since the publication of the "Origin of Species," by Charles Darwin, such a grouping has assumed a deeper significance. Since then there has been a general recognition of the probability that the types of living organisms present in the world have undergone change as part of the process we term Evolution. This process is thought to be gradual, and organisms that have only recently evolved from a common type are naturally still comparatively like one another. On this view resemblances between organisms become an indication of common descent in comparatively recent times (though the process of evolution must be thought of as taking place very slowly as compared with the brief lifetime of man), and natural affinity is expressed in the resulting grouping, which for this reason is often spoken of as a natural classification. All such classifications are of course but tentative, and are always undergoing modification with change in our views as to the significance of various resemblances between plants.

The task of the descriptive botanist has been greatly simplified by the method of naming plants introduced by Linnæus.

Plants that are in most respects like one another are grouped together as one kind or species, and if a number of species show sufficient characters in common they are grouped together as a genus. If a species seems sufficiently distinct from all others, then it is assigned a genus to itself. In any case, any plant should be adequately named for purposes of recognition if given both generic and specific names. This method of naming plants is known as the "binomial system." Thus the generic name of the broad bean is *Vicia* and the specific, *Faba*. In addition, it is usual to add the author of the name, so as to avoid confusion should the same plant be described and named differently by independent authors, or on the other hand, the same name be applied to different plants, so that the full name of the broad bean is *Vicia Faba* L., where L. means Linnæus.

This descriptive work was naturally of immense importance during the early phases of the science and is still proceeding at the present day. Any traveller in a tropical region, who pays attention to the flora, will be impressed by the wide scope for the taxonomic botanist, both from the purely scientific and from the economic point of view. We need to know the plants poisonous to stock, and to study means for their suppression, to know the forest trees so that the sources of valuable timber may be recognised and suitable steps taken for their propagation, regeneration, etc.

As an instance of the still existing need for taxonomic botany, the case of Chaulmoogra oil may be cited. This oil has been tried, apparently with considerable success, as a treatment for leprosy, but the seeds from which the oil is expressed are collected by natives from the jungle in Burma, Assam, etc., and the samples sold to the dealers from different localities vary so greatly that they are clearly not all seeds of quite the same kind of plant, and consequently the oil may vary in its suitability for this purpose. The reliable application of this treatment rests in part upon the exact determination of the species from which the seeds are collected. The task thus indicated possesses both difficulties and compensations, which may explain the fascination that botany may have for the plant collector. The botanist must visit the forests himself, often an expedition of months, involving careful preparations and unexpected hazards. He

must then make sure of the trees from which the native collectors are gathering their harvest ; he is then confronted with the task of obtaining samples of twigs, flowers and fruits, probably from a lofty tree in a dense jungle, where felling a tree may necessitate several days' work in tropical heat before his specimen is cleared from other vegetation and has space to fall.

Branches of Botany.

The attempt of the taxonomic botanist to classify plants according to their estimated degree of relationship naturally involves a close comparative study of the plants, when it immediately appears that the same structures or "members," e.g. roots, stems, etc., are present in slightly different guise throughout whole series of different plants. This comparative study of plant forms is often treated as a distinct branch of botany, **COMPARATIVE MORPHOLOGY**.

On the other hand, plants may be studied rather as living and growing organisms in which complex chemical changes are proceeding and important functions being performed. This branch of botany is known as **PLANT PHYSIOLOGY**, and has considerable significance to agriculturist, forester, and horticulturist, whose primary concern is the successful cultivation of living plants.

Both Morphology and Physiology involve some knowledge of the microscopic construction of the plant. To a large extent this resolves itself into the comparison of one structure with another, and is studied under the head of **COMPARATIVE ANATOMY**.

Whilst botanical study may thus be sub-divided into different branches, it cannot be over-emphasised that there is only one subject of study, the plant. Whilst this may be regarded from many points of view, there is one main purpose—the fuller understanding of the plant in all its aspects—and to this end it is important that anatomical structure, function, growth, development and form should all be fully utilised and brought to bear upon one another. Therefore in this elementary survey of the science of the plant, no formal distinction will be drawn between Morphology, Physiology and Anatomy, but information contributed from all these branches of our subject will be drawn upon as required in

order to complete our picture of the plant as a living entity. Our study will have to stop short of any close examination of the relation of the plant to its environment or habitat. These relations are very intimate, and their analysis throws much light upon the characteristic form and functional activities of particular plants, still more upon their distribution. The studies of plant distribution, geographic botany, have of recent years become associated with the study of the plant in its natural habitat—PLANT ECOLOGY.

Although it may be true that botany had its beginnings in the needs of man to select and cultivate plants for food or medicinal purposes, the study of the plant is well worth pursuing for its own intrinsic interest, and mankind will probably profit more, in the end, from studies of the living plant conducted without reference to practical considerations. In the following pages the aim will be the understanding of the plant itself, though occasionally it may prove opportune to point out that the conclusions gained from such a study of the plant seem to convey lessons of practical import.

Kinds of Plants.

Among plants there is a great multiplicity of form, and it is of great convenience if we can find some easily recognised characteristics which enable us to group them into classes for purposes of further study. As a first step in this classification, it is convenient to see whether the usual form categories of leaf, stem, and root (which are usually associated with the presence of a vein system) can be recognised. A second feature of great importance is whether the plants are multiplied by complex structures known as seeds, which are developed after flowering, or whether the multiplication is by much simpler structures, spores, which are not preceded by flowers. On such features as these the following classification may be outlined.

A CLASSIFICATION OF PLANTS.

- I. Plants with root, stem, and leaf, and containing a vein system : vascular plants.
 - A. Reproduced by seeds : seed or flowering plants.
 - (a) Seeds enclosed in a fruit case : Angiosperms.
 - (b) Seeds not enclosed in a fruit case : Gymnosperms.
 - B. Reproduced by spores : ferns.

II. Rootless plants, with or without simple leaves, reproduced by spores, and without a vein system : non-vascular plants.

A. Usually land plants, some show differentiation into stem and leaf, all have spore capsules : mosses and liverworts.

B. Plants without distinction into leaf and stem, no spore capsules.

(a) With green pigment, usually found in water : Algæ.

(b) Without green pigment, usually found on land : Fungi.

In the main, the following pages deal only with the group of flowering plants, since although these are the most highly organised and complex of all plants, they are also the most familiar.

CHAPTER II.

THE SEED AND GERMINATION.

Seed Plants.

Practically all our food plants, and therefore all plants of the farm and vegetable garden, as well as those cultivated in the flower garden, belong to the group of seed-bearing plants known as Angiosperms. As a result it is a familiar experience to collect and sow seed from such plants and thus reproduce their kind, but our familiarity with this process should not lessen our appreciation of its remarkable nature.

The seed sown by the grower has been produced inside some kind of dry or fleshy fruit, which has developed on the parent plant subsequent to flowering. Thus bean or pea seeds may be collected out of dry pods, or seeds may be separated from such fruits as the tomato and gooseberry, after which they are kept dry until the time of sowing. They are then planted in the confident expectation that the bean seeds will grow into new bean plants, the tomato seeds into new tomato plants, etc., and in this everyday procedure implicit faith is placed in certain general, though little understood, characteristics of seeds. The first characteristic is that a good seed, however small it may be, carries within it the potentiality to grow into the same general kind of plant as the one upon which it was produced. That is, in the tiny tomato seed must be packed all the hereditary characters which will produce in the next generation leaves, stems, hairs, flowers, fruits, etc., which we can recognise as identifying features of the tomato plant.

The second characteristic is that these potentialities are latent and only exhibit themselves if the seed is placed under certain conditions which will induce it to germinate and grow into a plant.

It is clear that the seed provides a natural starting-point for a preliminary study of botany, which is to be based mainly

upon the flowering plant, and we shall discuss first, therefore, the range of facts which present themselves when we study a few characteristic seeds.

The Broad Bean (Vicia Faba L.).

As the flowers of the broad bean plant wither, there will be seen in the centre a long, slender, green structure which will continue to grow and expand into the well-known bean pod. This pod, the fruit of the bean, is attached to the plant at one end by a short stalk, the former flower stalk, whilst the other end terminates in a curved point which served, whilst in the flower, to collect the pollen necessary for fertilisation before the formation of the seeds within the pod, a process which we shall be unable to discuss until much later.

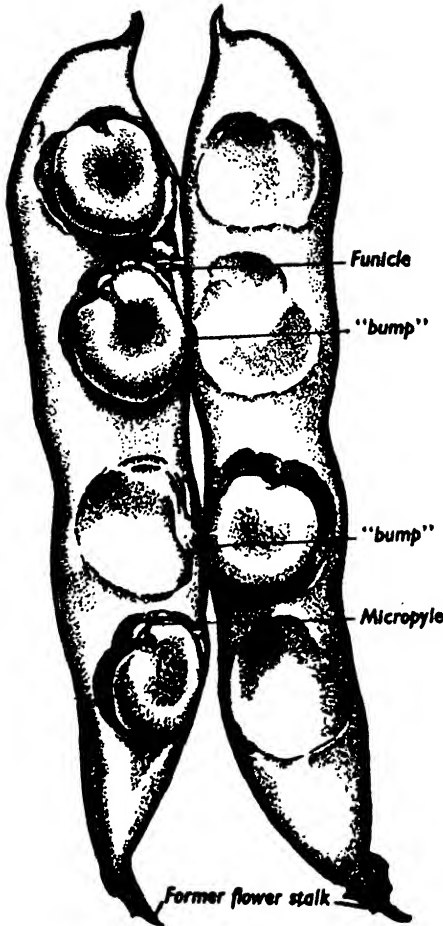


FIG. 1.—Pod of broad bean opened to show seeds ($\times \frac{1}{2}$).

The pod is a somewhat flattened, curved structure with a ridge along either narrow flank. With care, it may be split open along the more curved ridge and opened into two valve-like halves, in each of which a few seeds may be seen lying embedded in the soft fluffy lining of the fruit wall (Fig. 1). Each seed is

attached to the pod by a short stalk, known as the funicle, which is small and narrow where it joins the fruit wall,

but widens into an elongated disc where it joins the seed. When the fresh green seeds are taken out of the pod they alter considerably in appearance, becoming darker in colour, and wrinkling as they gradually dry. In this dry state the seeds are stored over winter. The seed is a large flattened structure of roughly oblong outline (Fig. 2); a conspicuous feature of one of the narrow sides is an elongated scar, the hilum, lying in a groove; this represents the region where the seed has

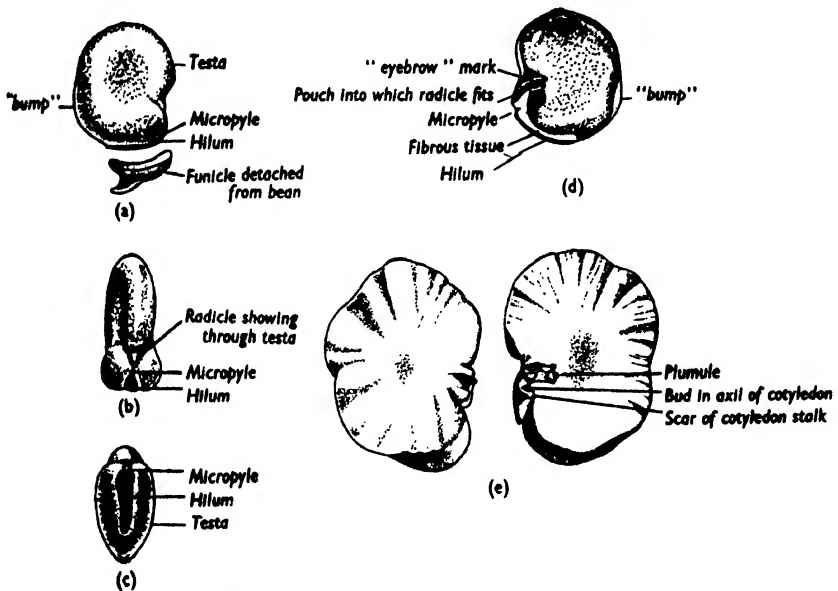


FIG. 2.—Seed of broad bean, (a), (b) and (c) different views of the seed and (d) inner surface of half the testa ($\times \frac{1}{2}$), (e) bean with testa removed and one cotyledon broken away ($\times 1$).

separated from the funicle (Fig. 2a). The tissues of the broad bean always darken upon injury, and this may be responsible for the black colour of this scar, which stands out as a conspicuous mark against the otherwise buff-coloured surface of the seed. About midway down one longer, narrower side, which is usually more curved in outline, a small, slightly discoloured bump is visible on the seed (Fig. 2d). This bump occurs at a point where the bean is in contact with the maturing pod (Fig. 1), but there is little reason to think this is a case of cause and effect.

The general buff-colour of the seed is due to a skin or testa,

which is wrapped closely around a white mass within. This testa adheres closely when dry, but can readily be pulled off after soaking the seed in water. The surface becomes very wrinkled as water is absorbed, since the testa swells sooner than the contents, but gradually the interior mass also takes in water, and by the time the seeds have been soaking for about twenty-four hours, the testa is usually stretched smooth by the swollen contents. In this condition one other feature may be made out, namely, that if the seed is squeezed, water and air bubbles are seen to come out from a small, slit-like hole, the micropyle, which lies at one end of the hilum (Fig. 2*b, c*). If the testa is removed by cutting it open on the side farthest from the micropyle it will come off in one piece, and will be found to consist of a thin, smooth outer layer with a waxy appearance, and a more fibrous lining which is especially thick under the hilum. Beyond the hilum, this fibrous tissue extends over the region where, on the outside, the micropyle could be seen, so that this little opening does not give a free passage through the skin, but is blocked on the inner side by this felted fibrous inner layer (Fig. 2*d*). Above this again the fibrous layer is folded as a pocket around the radicle. When the seed is squeezed, water and air bubbles must be driven out of the micropyle from this rather absorbent inner coat. The central mass with the testa removed can be opened up, rather like a book, into two thick lobes, the cotyledons, which are hinged together by a small structure which is conical at one end, and which forms the radicle; at its other end, the plumule curves in between the cotyledons, and may be seen (with the help of a pocket lens) to have at least two small, scale-like structures upon it (Fig. 2*e*). In addition to these more obvious features, two small "eye-brow"-like marks are present on the "shoulders" where each cotyledon narrows to a short, stalk-like region to join the radicle, and these are seen to correspond with similarly shaped and slightly darkened marks on the inside of the testa. The explanation of these appears to be that, during development, the growing tissues of the embryo have been pressing against the testa, and the slight bruising has produced the usual discoloration. Another feature which may be seen upon close observation when a cotyledon is broken away or bent downwards, is a very tiny bud in the angle between the cotyledon and the plumule.

Seedling from Seed.

Even in such a large type as the broad bean, the structures within the seed are very small, and the interpretation of the nature of the various parts becomes much easier if their further development is watched over a period of a few weeks. If the seeds are kept damp in soil, or sawdust, the contents continue to take up more water and to enlarge until, after a few

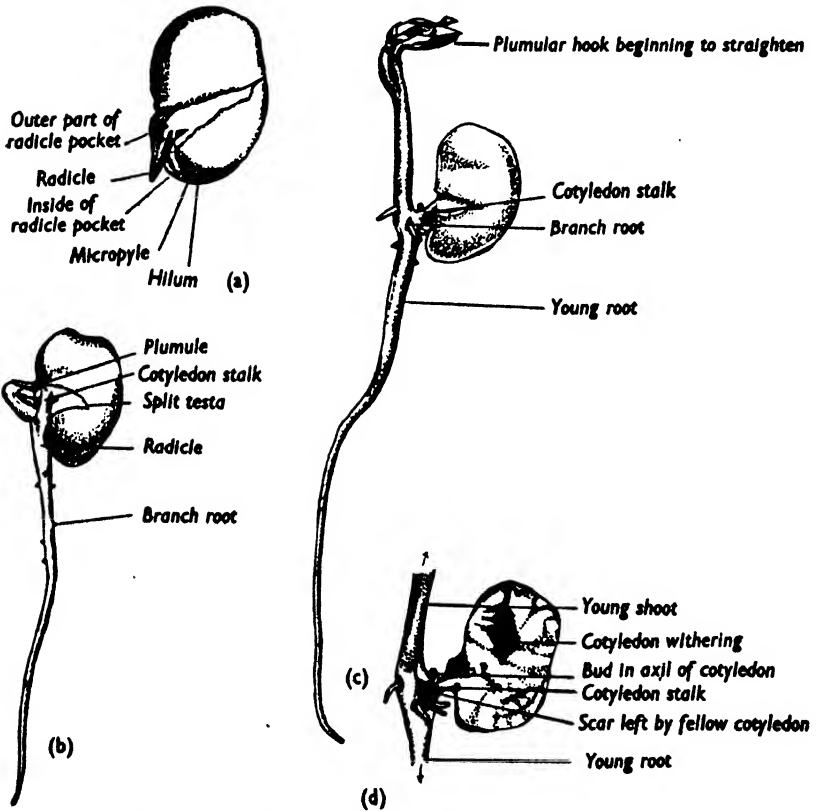


FIG. 3.—Broad bean seedlings showing stages in germination (*a* and *d* $\times \frac{1}{2}$, *b* and *c* $\times \frac{1}{3}$).

days, the testa can accommodate them no longer. The tearing of the testa takes place in the neighbourhood of the radicle, which usually forces its way through the base of the pocket in the testa (Fig. 3*a*); but not infrequently the testa tears across just above the junction of the radicle and cotyledons. Once formed, the tear rapidly extends and the

plumule emerges from between the cotyledons as a sharply-hooked structure of which the scale-clad apex grows up as the seedling shoot (Fig. 3*b, c*). In the light, this shoot soon becomes green, and leaf-like structures are unfolded from it. The first two leaves, which are identical with the two tiny, scale-like lobes which could be recognised on the plumule, are carried up by the elongation of the epicotyl, the region of the shoot axis above the cotyledons, and also become separated from one another by elongation of this axis. Although these two scale leaves grow considerably, they always remain small and different in form from the third and subsequent leaves which unfold as the shoot grows.

Scarlet Runner Bean (Phaseolus multiflorus Willd.).

The beans are formed in pods as in the case of the broad bean, but the funicle is shorter and is attached to the middle of a long side of the bean in a more symmetrical position. The seed coat is pink in colour, strongly mottled in black, especially on the side away from the funicle (Fig. 4*a*).

When the structure is examined in detail, it is found to be essentially the same as in the broad bean. To one side an oval scar indicates the position of attachment to the funicle, whilst just above this is a conspicuous micropyle. At the other end of the scar there are two little raised structures and, although these are constant features of this particular type of seed, their significance is not apparent (Fig. 4*b*).

When the testa is carefully removed from a soaked seed, we find that the bulk of the structure inside consists of the two thick cotyledons, which are attached to the short axis consisting of a radicle and plumule. The radicle projects a little (Fig. 4*c*) and fits into a pocket in the fibrous lining of the testa, which, as in the broad bean, points directly towards, but has no open communication with, the micropyle. When the two cotyledons are prised apart the plumule is seen curved in between them. In this case it is very clear that this structure is a small leafy bud, for the two outermost leaves are already well formed, and it is possible, even without a lens, to see their folded outline and the pattern of the veins (Fig. 4*d*).

When the runner bean germinates, one usually finds that the hypocotyl, or region of the axis just below the cotyledons,

does not elongate appreciably, so that the cotyledons remain in the soil, whilst the epicotyl, or region of the axis above the cotyledons, develops and carries the leaves up into the light and air (Fig. 4*e*). Occasionally the hypocotyl may elongate slightly and carry the cotyledons above the soil; under these conditions they open out from one another and turn green, looking like a pair of thick fleshy leaves.

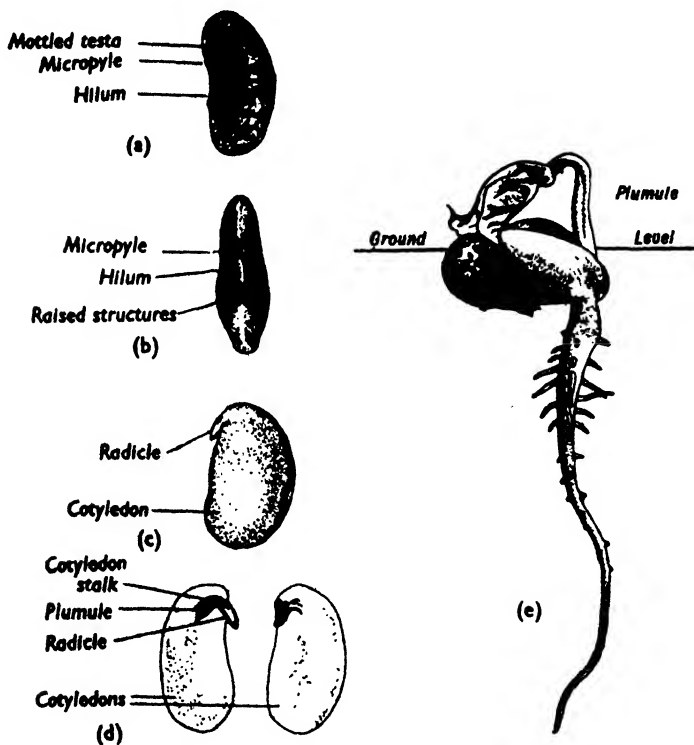


FIG. 4.—Seed of runner bean, (a) and (b) views of whole seed, (c) with testa removed, (d) cotyledons separated, (e) seedling ($\times \frac{1}{3}$).

In another type, the French or kidney bean (*Phaseolus vulgaris* L.), it is normal for the hypocotyl to elongate and the cotyledons are green and more leaf-like. Cotyledons which remain below the ground are described as hypogeous and those which come above the ground as epigeous, but which of these two modes of growth prevails depends upon the degree of elongation of the hypocotyl, and in the runner bean either mode may occur. On the shoot of the runner bean, the first

two leaves to expand are the ones already seen to be well-developed on the plumule in the seed. They are borne opposite to one another at the same level on the shoot and, unlike the first leaves in the broad bean, are relatively large heart-shaped structures, each with its own stalk. The subsequent leaves are borne singly at either side of the stem and also differ in shape from the first two.

The pea (*Pisum sativum* L.) is another seed which is very similar in construction to the broad bean, differing chiefly in the more rounded form of the seed. The broad bean (*Vicia Faba* L.), the runner bean (*Phaseolus multiflorus* Willd.), the kidney bean (*Phaseolus vulgaris* L.), and the pea, are all seeds of very much the same type, and as similarities also exist between the plants produced from the seed, they are regarded as related to one another and are grouped together in any system of classification. They are all placed in one family, the Leguminosæ, but as the runner bean and the kidney bean show especially close resemblance to one another, these two species are placed within the same genus, *Phaseolus*.

Germination.

The development of the seedling from the seed is spoken of as germination. During this process the testa simply rots away, but the minute plumule gives rise to the shoot and the radicle to the root, whilst the cotyledons slowly shrivel and become softer and more wrinkled. These changes only occur under suitable conditions, as the following simple experiment will show :—

Four glass jars A, B, C, and D are taken. Into each is put some glass wool and a small handful of dry peas. A is left dry. Into B is poured sufficient water to cover the peas, and into C and D, a little water. D is put in a cold place, and A, B, and C in a warm room. Each jar is covered with a glass plate to prevent much loss of water. If these jars are watched over a period of time, it is clear that the seeds in A fail to germinate owing to the absence of water. In B and C, where water is present, growth starts, but a difference is soon apparent between those merely in damp air in C, which continue to develop, and those which are completely covered with water in B, which soon fail to make any further progress. The reason that obviously suggests itself for this difference is

that those covered with water have not enough air. The point may be further tested by growing some peas under running water. The dissolved air is then continually renewed with the water and the peas germinate quite readily. Comparison between C and D, which are under like conditions except for C being in a warm place and D in a cold, will probably show such differences in the degree and time of germination as to justify the conclusion that a suitable temperature is an important factor.

Entry of water is obviously an essential preliminary to germination. The water evidently penetrates within the testa, for the inner part of the seed swells, thus smoothing out the wrinkles in the enlarged seed coat and finally rupturing it. The question naturally arises as to how the water penetrates the seed—does it enter mainly through the micropyle, from which water and air bubbles emerged when the soaked seed was squeezed, or does it enter through the testa as a whole? Simple experiments may be carried out to throw light upon this point. The micropyles of dry seeds may be blocked with melted wax, and the gain in weight in these seeds compared with the gain in weight of others without the micropyle blocked, after all have been immersed in water for about a day. Alternatively seeds may be suspended by wire clips with all but one end of the seed in water, and the gain in weight compared when the micropyle is above or below water. It is always found that beans vary very considerably among themselves in the rate at which water is taken up, but if a sufficient number of beans is used for comparison in either of the above ways, the results show beyond doubt that water enters all over the surface of the testa, though it may enter rather more quickly at the end with the micropyle. Thus in Table I the gain in weight of individual beans with the micropyle immersed ranges from 82 to 131 per cent. ; of beans with the micropyle out of water, from 36 to 106 per cent. When batches of beans were taken the irregularities were still apparent, the percentage increase with the micropyle immersed varying from 69 to 124 per cent. ; with the micropyle out of water, from 54 to 75 per cent. Evidently when the region around the micropyle is immersed, water does enter rather more readily, but it must also enter through all regions of the skin, and at very different rates in different beans.

TABLE I.

BROAD BEANS—INTAKE OF WATER.

Individual weights.

(All weights in grams.)

Seed.	Initial weight.	Weight after 3 days.	% increase in weight.	
<i>I. Suspended with the seed under water, except the end opposite the micropyle.</i>				
<i>a</i>	2.070	3.838	85	seed swollen
<i>b</i>	1.506	2.850	89	fairly hard
<i>c</i>	2.052	4.267	131	radicle just through
<i>d</i>	1.490	2.813	89	seed swollen
<i>e</i>	1.517	3.173	109	radicle just through
<i>f</i>	1.604	3.388	111	radicle through
<i>g</i>	1.755	3.559	103	radicle just through
<i>h</i>	1.648	3.018	82	seed wrinkled on one side
<i>II. Suspended in water, with the micropyle just above surface of water.</i>				
<i>a'</i>	2.296	3.632	59	seed fairly hard
<i>b'</i>	1.638	3.173	94	seed swollen
<i>c'</i>	1.655	3.405	106	seed swollen
<i>d'</i>	1.838	2.504	36	seed wrinkled
<i>e'</i>	1.723	2.606	51	seed wrinkled
<i>f'</i>	1.932	3.895	102	seed very swollen
<i>g'</i>	1.524	3.017	98	radicle well through
<i>h'</i>	1.771	3.145	78	seed swollen

Seeds weighed in groups.

Set.	No. of seeds.	Weight.	Weight after 3 days.	% increase in weight.
<i>Seed under water, except the end opposite the micropyle.</i>				
1	8	17.52	34.74	98
2	8	13.50	27.30	102
3	6	9.22	15.56	69
4	6	8.31	18.71	124
<i>Micropyle above water.</i>				
1	8	13.29	23.27	75
2	8	14.27	22.02	54
3	6	10.33	17.98	74
4	6	9.52	15.89	66

When students have been carrying out this experiment, the gain in weight of individual beans in two days has sometimes been astonishingly small with the micropyle out of water—not more than 3 per cent. in one case, 19 per cent. in another. In clover, another plant of the same family as the bean, this phenomenon is very marked. Many clover seeds fail to take up much water for months or even years although they are under suitable conditions for germination, and in such cases the entry of water can be facilitated by scratching or cracking the skin. The outermost surface of the testa of the broad bean or clover is smooth and shiny and is of a greasy, waxy nature which does not readily wet, or absorb water. In the bean the layer is usually sufficiently thin and scanty to permit the entry of water into the more absorbent layers below, but in the so-called “hard” seeds of clover this layer seems to be so thick and continuous as to delay germination for an indefinite period. For the same reason the seed coat of some kinds of sweet peas are cracked as a regular practice before planting. More detailed experiments on seeds of *Vicia* and *Phaseolus* have shown that the micropyle is responsible for some 20 per cent. of the water absorbed during the first twenty-four hours. This phase of entry is important as it causes swelling of the testa and the resulting stretching and cracking of the waxy surface layer then permits more ready absorption over the whole surface.

The Seed Coat as a Semipermeable Membrane.

Other simple experiments show that although water enters the seed through the seed coat at the micropyle or elsewhere, substances dissolved in the water by no means necessarily enter through the seed coat too. Thus, if a drop of aqueous methylene blue is placed on the inner surface of a piece of testa removed from a dry bean, and the testa is then floated like a boat on a dish of water, none of the dye is seen to diffuse into the water.

If the experiment is repeated by taking a fresh piece of testa and replacing the drop of methylene blue by a few grains of sugar, these are seen to dissolve rapidly. If a second piece of testa with sugar is prepared at the same time, but not floated on the water, the sugar remains dry, which makes it

clear that in the case of the floated testa the water actually penetrated the seed coat and did not condense from the moisture in the air. In this experiment, the sugar being colourless, it is not easy to determine whether it has also been diffusing through the seed coat into the water, but a third experiment gives evidence on this point.

A rubber stopper, with a wide hole bored through it, is cut through transversely. Between the severed pieces, a portion of the testa from a soaked bean is inserted so as to cover the hole completely. A glass tube (with the edges ground smooth with sand-paper) is fitted in the hole so as to be in contact with the inner surface of the piece of testa, and the two halves of the stopper are then fastened firmly together. The stopper is then immersed in a beaker of water, care being taken that no air bubbles prevent the water from making contact with the testa. Sugar solution is poured into the tube and its level marked. As the level of the liquid in the tube subsequently rises, the inward movement of water is evidently much more rapid than any outward diffusion from the sugar solution. These experiments show that the testa is behaving as a semipermeable membrane, as the solvent, water, is moving across it much more readily than the solutes, the sugar and the dye. Water will diffuse through such a semipermeable membrane according to the osmotic gradient. Entry of water into the intact bean probably takes place by a similar mechanism to that involved in these simple experiments with detached pieces of testa.

This statement may be amplified for the non-chemist who, however, must turn to the chemists for a fuller and more exact explanation. If a soluble substance, such as sugar, is placed in water it will inevitably "dissolve," that is, it will disappear from sight and diffuse as a "solute" through the "solvent" water until its concentration is uniform throughout the liquid. If the water in the experiment occupied two compartments, separated only by a "semipermeable" membrane, and if the sugar was placed in the water only in one compartment, then, as the sugar dissolved it would be unable to diffuse through the membrane. The water on the other side of the membrane would then be drawn across the membrane by the tendency for the sugar to dissolve in it, i.e. by the affinity between sugar and water (Chapter XVIII). This force draw-

ing the water across the semipermeable membrane into the sugar solution is termed the osmotic pressure of the solution and naturally is proportional to the concentration of the solution.

Dry bean seeds in the autumn of the year of harvesting lose about 15 per cent. of their original weight on drying in a steam oven at a temperature of 100° C., so that about 15 per cent. of their weight appears to consist of water. If similar seeds are soaked for twenty-four hours in water, they frequently take up about 150 per cent. of their original weight of water, and this rapid increase certainly suggests that the contents of the seed have a great affinity for water and draw it in just as the sugar solution did when separated from the water by the testa. We can see also that the entry of water will depend upon a good contact between water and seed coat on the outside of the seed, and between seed coat and contents on the inside. This is probably the main reason why germination is carried out by the gardener in a seed bed of finely divided soil, which is pressed down firmly after the seeds are sown. Thus even small seeds are planted so as to be in contact with the films of water which surround each soil particle. In a loose soil, little contact is made between soil particles and testa, and unless the soil is flooded with water (when air will be deficient), the films of water from the soil particles will not completely cover the testa and entry of water will be very slow. Similarly, as the water has to be drawn in through the testa into the seed, it is clear that a close contact between the contents of the seed and the testa is essential.

Another practical instruction in planting seeds is to avoid treating the seed bed with artificial manures such as nitrates, although these are usually regarded as substances which promote rapid growth. But we see now that these soluble substances, through increasing the concentration of the soil solution, would delay the entry of water into the seed, and it is easily possible to demonstrate by a simple trial that the addition of such soluble manures to the seed bed may materially retard the first stages of germination of the seeds.

Significance of Germination.

As germination proceeds, water continues to be absorbed by the developing seedling in considerable quantities, and

actual weighing experiments show that the increase in bulk of the seedling is due almost entirely to the intake of water. In Table 2 fresh weights and dry weights of the pea plant are given at intervals after sowing (the figures being in each case an average for a number of plants). In these weighings the testa is excluded as it gradually rots away in the soil, and obviously plays little or no further part in the development of the seedling plant from the seed. Comparing the fresh weights, it is seen that the plants rapidly increase in weight, so that, starting with a dry seed weighing 0.289 gram, the subsequent weighings after periods of twenty-one and twenty-eight days respectively are 2.38 grams and 2.49 grams. That this increase is due almost entirely to intake of water is shown by drying the plants at 100° C., so as to drive off all moisture, and taking the dry weights, which throughout the period remain between 0.21 gram and 0.382 gram. We cannot, however, consider this simply as an expansion of the parts of the seed by water, since the whole appearance and relative proportions of the parts are altered during the period of germination. We have seen how the parts which were least conspicuous in the seed, the radicle and plumule, become the most conspicuous as the branched root and leafy shoot of the seedling, whilst the cotyledons which formed the main mass of the seed gradually become thinner and shrivel away during germination, never making much growth, even in such cases as the kidney bean where they come above the ground and turn green. This observation is placed on a more definite footing if we turn again to Table 2 and follow the changes in fresh weight and dry weight during growth. Very few weights of the fresh tissues are available in this table as it is difficult to collect the plants, remove the soil, etc., and prepare them for the balance, before the loss of water from them has made the weight meaningless as a measure of their original fresh weight. The results of some earlier weighings are therefore given in Table 3, in which the peas were grown in pots in a greenhouse near the laboratory. Under these conditions the plants were not grown to maturity, but more data are available for the first few weeks, and again all figures represent averages from the weighing of a number of plants.

Utilising Table 3 then for the earlier data, it is seen that in a seed with an average weight of 0.26 gram, 0.23 gram is

TABLE 2.

PEAS—WEIGHTS AND DRY WEIGHTS (in grams).

Wt. of one stock seed (without testa)	= 0.2890	}	Average of 50.
Dry wt. of one seed (without testa)	= 0.2290		
" " plumule	= 0.0008		
" " radicle	= 0.0011		
" " pair of cotyledons	= 0.2280		

Planted June 8th, 1931 : Grown in LIGHT.

(Subsequent weights are averages calculated from batches of six plants.)

Date.		Fresh wt.	Dry wt.	
June 22	shoots (limp) roots cotyledons		0.030 0.023 0.164	Shoots 6 ins. high, hooked Total D.W. = 0.217
June 29	shoots roots cotyledons	2.38	0.125 0.046 0.039	Leaves spreading Total D.W. = 0.210
July 6	shoots roots	2.49	0.244 0.138	Total D.W. = 0.382
July 13	shoots	7.64	0.785	
July 20				
July 27	shoots	24.50	2.655	Flowering
Aug. 4	shoots	34.46	4.149	Occasional pods
Aug. 11	shoots	26.35 greatly delayed in transport	3.596	About 2 pods per plant, lower leaves badly eaten by slugs
Aug. 18				
Aug. 25	shoots	63.34	8.586	
Sept. 11	shoots	68.07	7.782	Some pods fairly full, leaves badly eaten
Sept. 9				
Sept. 15	shoots	83.43	13.541	Pods filling well

retained after drying at 100° C., so that there is only some 12 per cent. of water present, and the dry weight of the cotyledons, plumule, and radicle (respectively 0.21, 0.0008, and 0.02 gram) must represent fairly accurately their original weight. After eight days in the soil the weight of the cotyledons has increased to 0.71 gram, and that of the shoot and root to 0.40 and 0.35 gram respectively, a very much bigger gain proportionately than in the cotyledons. But after twenty days, the weight of the cotyledons is actually diminish-

TABLE 3.
PEAS GROWN IN SOIL IN GREENHOUSE.
(All weights in grams.)

		Original seed.	Seedlings.			
			8 days	20 days.	27 days.	33 days.
Shoots	Fresh weight		0.40	1.53	2.40	3.13
	Dry weight	0.0008	0.035	0.12	0.75	0.26
Roots	Fresh weight		0.35	0.85	0.73	0.97
	Dry weight	0.02	0.02	0.035	0.04	0.04
Cotyledons	Fresh weight		0.71	0.47	0.23	0.40
	Dry weight	0.21	0.13	0.03	0.017	0.01
Whole plant (except testa)	Fresh weight	0.26	1.45(i)	2.85	3.36	4.51 (ii)
	Dry weight	0.23	0.18	0.19	0.80	0.31
Date (1930)		July 2	July 10	July 22	July 29	Aug. 6

(i) Leaves partly expanded.

(ii) Lower leaves decayed and falling.

ing (0.71 to 0.47 gram), whilst shoots and roots are still increasing very rapidly in weight (shoots 0.40 to 1.53 grams, roots 0.35 to 0.85 gram).

A study of the dry weights further shows that all the original increase in weight of the cotyledons was due to intake of water, the dry weight having diminished in the first eight days from 0.21 to 0.13 gram, when the fresh weight had actually increased to 0.71 gram. On the other hand, in the case of shoots and roots (paying attention only to plants grow-

ing in the light), the dry weights are increasing substantially (shoots from 0.0008 to 0.12 gram, roots from 0.02 to 0.35 gram). One curious result worth especial emphasis is that all through the first twenty days, when young plants of quite a considerable size are produced, their lower leaves well expanded and their roots forming a vigorously branched system, the dry weight of the plant as a whole actually diminishes from 0.23 gram originally to 0.18 gram after eight days, and 0.19 gram after twenty days.

Similarly, in the peas grown in the open in 1931, the original dry weight of one seed was 0.229 gram. After twenty-one days the average dry weight was still only 0.21 gram.

Germination, therefore, has involved not only an intake of water, but also a total loss of solid matter, accompanied by a transfer of solid matter from the cotyledons to the developing shoot and root. With these facts before us, we may form a more generalised concept of a seed of this type. It consists essentially of a plumule, the young upwardly growing leafy axis, and the radicle, the young downwardly growing root. Between these two growing regions, which always tend to diverge further from one another, there is present a food supply in the cotyledons from which food moves to both growing centres. These parts together constitute the embryo plant, which in the seed is enclosed in a testa.

The Seedling and Loss of Dry Weight.

Table 3 shows that although the increase in weight of the shoot and root of the peas is due largely to intake of water, a transfer of solid substance from the cotyledons to the shoot and root is also involved. Observation of the seedling shows too that new structures have appeared, in the form of additional leaves on the shoot and branches on the root, and it is natural to conclude that the solid substances which have entered the shoot and root from the cotyledons have contributed to the building up of these new parts.

The movement of solid substances in the plant has been deduced from observations of "dry weight." This is the weight of the plant tissue after drying at 100° C. until its weight is constant. Under these conditions water is lost, but various easily decomposable solid substances are left. If this dry

plant material is burnt in air, the tissue being heated to a red heat in a crucible until the weight of the residue remains constant, a very large bulk of the tissue will be lost, and only a small percentage of the dry weight will remain in the form of ash, consisting of mineral or inorganic substances. Thus from an analysis of a sample of fifty pea seeds, it is found that the total dry weight of one seed (with testa removed) is 0.2128 gram, the ash left after burning 0.0089 gram, so that the ash forms 4.2 per cent. of the dry weight. In the same way, two different samples of broad beans showed ash as 5.01 per cent. and 5.7 per cent. respectively of the dry weights.

The fate of the substances that have disappeared can be elucidated by heating the dried plant material, composing the dry weight, in a hard glass test tube with a delivery tube leading into lime water. Water will be seen to condense in the cooler part of the test tube and delivery tube, whilst large quantities of carbon dioxide will precipitate the lime water. The carbon dioxide and water have been released from the dry material, evidence that it contains carbon combined with hydrogen, probably also with oxygen. Chemical analysis usually shows that some 50 per cent. of dry plant material consists of carbon and therefore is composed largely of carbon compounds. Living organisms are always constructed of such carbon compounds, which are therefore often spoken of as organic compounds, and are also the main constituents of the foods which we assimilate to build up our own living tissues.

Seeds thus contain food reserves and indeed form some of our main sources of certain food substances, e.g. starch from cereals, proteins from beans and peas, fats from nuts, etc. Food reserves are present in the cotyledons of the pea, and when plumule and radicle grow at the expense of the dry weight of the cotyledons, we are witnessing the utilisation of these foods for the growth of the plant. But to the chemist, familiar with the nature of such substances as starch, protein, and fat, this raises the problem of the transference of these substances from the cotyledons to the growing plumule and radicle. They are all insoluble substances, and it is impossible to conceive of their being moved about in the tissues in any but a soluble form. However, one characteristic of all these commonly occurring types of food reserves, is that they are capable of being broken down to simpler, water-soluble sub-

stances by a type of chemical reaction involving the addition of water and consequently known as hydrolysis.

Thus we find—

starch plus water giving sugars,

proteins plus water giving soluble peptones and amino-acids,

fats plus water giving fatty acids and glycerol.

This breaking down by water, or “unpacking” of the food reserves, is undoubtedly one of the first effects of the entry of water into a seed. So long as the seed remains dry, the food reserves remain unchanged and so are not available for the growth of the embryo, and the seed, although it may remain alive, does not germinate. Once water enters and the reserve foods become changed into soluble forms, germination begins and must continue or death ensues, for the soluble substances must be used in growth or further changes take place and they are gradually lost from the plant, usually in some process of decay.

If, however, the relevant figures are studied in Tables 2 and 3, it will be seen that all the substance lost from the cotyledons, as indicated by this loss in dry weight, cannot be accounted for in the observed increase in dry weight of root and shoot. Thus from Table 2, by 22nd June the cotyledons have lost $0.228 - 0.164 = 0.064$ gram, whilst shoot and root have gained by that date respectively $0.03 - 0.0008$ and $0.023 - 0.0011$ gram, a total gain of about 0.05 gram. Similar results may be deduced from the data in Table 3. Furthermore, study of the dry weight of the whole plant as compared with that of the original seed during this period will show that the plant as a whole is losing dry weight. In Table 2 the average original dry weight, 0.289 gram, falls after fourteen days to 0.217 gram, after twenty-one days to 0.210 gram. In Table 3 an original dry weight of 0.23 gram becomes in eight days 0.18 gram, in twenty days 0.19 gram. This loss, in one case 0.073 gram out of 0.289 gram, or about 25 per cent., is much too high to be accounted for by loss of ash and must represent an actual loss of organic substance. One of the most fundamental characteristics of organic compounds is that they readily burn to carbon dioxide and water, releasing energy in the process, and in all probability they are being “burnt,” though very slowly, during the process of germination. Energy is required for any constructive process, and so in the

present instance energy is necessary for the work done in transferring food from the store to the growth centres and there building it up into new structures. Our dry weight tables therefore tell us that in one case, $0.289 - 0.217 \text{ gram} = 0.072$ gram of organic matter have been burnt to supply the energy to transfer about 0.05 gram of substance from the cotyledons and build them up into new shoot and root tissues.

The burning of organic matter that takes place in living organisms is a very slow process when compared with ordinary combustion and proceeds at relatively low temperatures. It is often spoken of as respiration, and the contrast between it and combustion may be brought out to some extent by simple experiments.

In all processes of combustion organic carbon compounds are being burnt. They are combining with the oxygen of the air with the formation of carbon dioxide and water vapour, and at the same time energy is being released. A simple example of such combustion is supplied by a smouldering cigarette, air being drawn through the burning tobacco leaf into a flask which contains lime water. The rapid production of a chalky precipitate in the lime water, much more rapid than when the air is drawn through the unlit cigarette, shows that carbon dioxide is being freely produced by the combustion of the cigarette. When the cigarette burns, we start the process by applying a flame. Until thus heated to a high temperature, the process of oxidation or combustion does not start. It then proceeds so rapidly that enough energy is produced to keep the cigarette glowing. If a drop of water is placed on the glowing end the temperature may be lowered so quickly that the oxidation ceases and the cigarette goes out, so that the rapid combustion of dried leaves is hindered by the addition of water. It can readily be shown that germinating peas also release carbon dioxide in considerable quantities, but dry peas do not, so that this process is associated with germination, which, we have seen on page 14, only proceeds in the presence of air and water. The relatively slow combustion of the substances composing the dry weight of the peas not only depends upon air, but is facilitated by the presence of water. Light may be thrown upon this fact by another simple experiment in which the release of carbon dioxide is accelerated by the addition of water to baking powder. This

is not a case of combustion ; the baking powder consists of two solid substances, sodium bicarbonate and tartaric acid, both soluble in water, which, when dissolved, immediately intermingle and react. The water here facilitates the reaction by bringing the substances into solution and thus into intimate contact.

Similarly, dry seeds respire extremely slowly. When water is absorbed processes of hydrolysis are initiated which produce soluble organic substances. These then meet dissolved oxygen and a comparatively slow process of oxidation occurs, which we distinguish as respiration. This process will be examined more closely at a later stage (Chapter XX), but it is evidently responsible for the loss of dry weight in germination of the seed, and for the provision of the energy necessary for growth.

During the early stages of germination in all seeds we find that the production of new tissues proceeds at the expense of the food reserves in the seed, which are partly used in these constructive processes, and partly burnt or respired away to supply the necessary energy.

It is obvious that this process cannot alone continue to affect dry weight during growth, or the new plant derived from the seed would contain less material than the seed itself. In actual fact, of course, any normal plant increases in substance, and in time bears a number of seeds of equivalent weight to that from which it has been produced. This must involve a considerable increase in the dry weight of the plant, an occurrence which is indicated by the figures for the later stages of growth of the peas in Table 2. This increase in dry weight is of great importance when studying the transition from the seedling to the adult plant (Chapter IV), and will be examined after a wider range of examples of seeds has been passed in review.

CHAPTER III.

SEED AND GERMINATION (*continued*).

Other Examples of Seeds.

Our first examples of seeds all possess two growing centres, the plumule and radicle, which develop respectively into the shoot and root of the new plant ; situated between these are the two cotyledons, containing the food supplies. These general characteristics hold for many seeds, but on reviewing a wider range of examples, some very interesting differences are found. A few selected examples will now be described which will permit the recognition of some of the more significant differences, and as the observations will have been made upon specific cases, the facts recorded will always

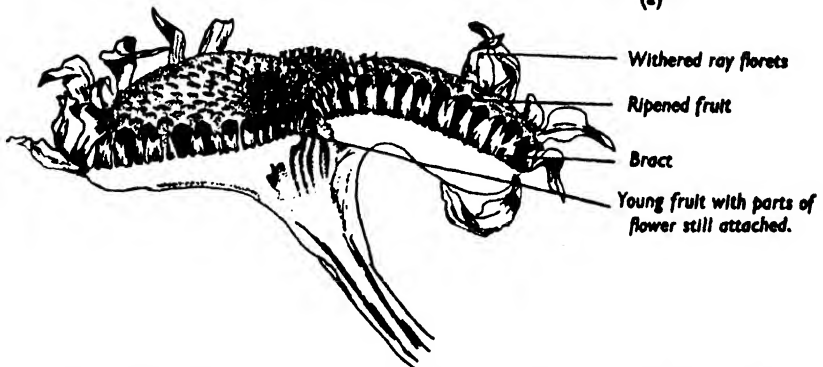
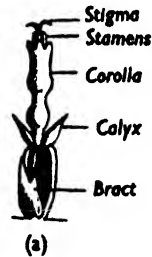


FIG. 5.—Half a head of sunflower fruits ($\times \frac{1}{2}$), (a) young fruit with the flower parts still attached ($\times 1$).

remain, however generalisations drawn from them may come to be regarded in the light of fuller experience.

At this point it is important to emphasise that the figures and descriptions given here are not intended to take the place

of actual examination of specimens. It is hoped that with facts drawn from observation to check and supplement the descriptive statements in these pages, the reader will be in a position to examine critically the validity of any generalisations.

In the sunflower (*Helianthus annuus* L.), the fruits develop in large numbers from the yellow, central part of the large head of flowers, collectively known as a sunflower (Fig. 5). The individual fruits are relatively small and do not open to liberate the single seed, so that the black, or black and white, streaked structures sold as sunflower "seeds" (Fig. 6a) are more correctly termed fruits. When the flower head is first

gathered, many of the fruits may still be surmounted by the withering remains of the yellow tubular parts of the flowers (Fig. 5a). When these fall off, two scales, also part of the flower, may persist for a short time. Eventually the position of attachment of the rest of the flower can only be recognised as an oval scar at the broad end of the somewhat flattened

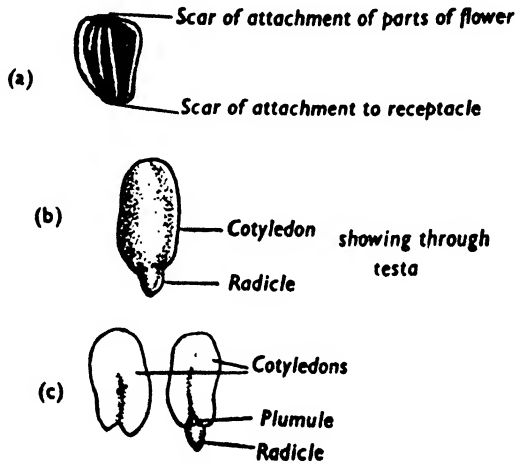


FIG. 6.—(a) Sunflower fruit ($\times 1$), (b) with fruit wall removed; (c) with testa removed and cotyledons separated ($\times 1\frac{1}{2}$).

fruit, which tapers at the other end to its point of attachment to the flower head (Fig. 6a). When the fruits are examined, either dry or after soaking, the hard fruit wall or pericarp can be removed, showing the seed inside enclosed in a thin, skin-like testa (Fig. 6b). After taking off the testa, the inner mass is found to consist of a conical radicle at the tapering end, above which are attached the two relatively thick cotyledons. On separation of the latter, it is just possible, with the help of a lens, to distinguish a very small, two-lobed structure, which on germination proves to be the plumule (Fig. 6c). The essential parts are the same as in the broad bean, the main

difference being that here the parts are much more symmetrical and the plumule very much smaller.

On germination the radicle emerges first, but this is followed by elongation of the hypocotyl, which causes the cotyledons, still enclosed in the seed and fruit coats, to be carried up above the soil level. After a time the coat is thrown off and the cotyledons separate from one another and expand into the first two green, leaf-like structures on the plant. The plumule is very slow to develop and shows little growth until the cotyledons are expanded, when it gradually elongates into the seedling shoot. The cotyledons are thus more definitely and regularly epigeous than those of the French bean, they are thinner and more leaf-like than in that type, but

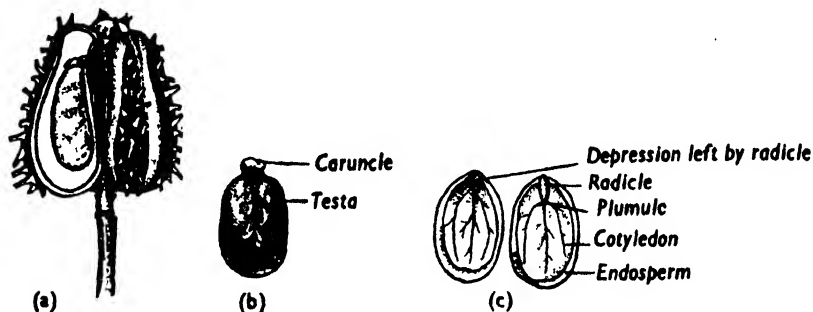


FIG. 7.—*Ricinus*. (a) Fruit with part of wall removed to show attachment of seed, (b) single seed, (c) seed with testa and part of endosperm removed, and split between cotyledons ($\times 1$).

they still represent the food supply in the seed, which is drawn upon in the early stages of germination.

In the castor oil (*Ricinus communis* L.), we are again dealing with a case where true seeds are liberated from a fruit. The fruit is somewhat oval in form and consists of three compartments, in each of which one or two seeds are suspended (Fig. 7a). The seeds are slightly flattened, oval structures, with a dark brown testa mottled in a lighter shade. At one end there is a curious, light coloured outgrowth, the caruncle (Fig. 7b), which is evidently continuous with the tissues of the seed coat. The small attachment of the funicle can just be seen close beneath the caruncle. The micropyle is overgrown by the caruncle, but, on removal of the latter, a black triangular point is revealed, at the apex of which the micropyle is situated.

Although a conspicuous feature of the castor oil and some other seeds, e.g. gorse (*Ulex europæus* L.), and broom (*Cytisus scoparius* Link.),* the caruncle does not appear to be an essential part of the seed. It was represented in *Phaseolus* by the raised structures near the hilum. One suggestion as to any effect it may have on the plant as a whole is that ants are said to carry off gorse seeds so as to use the sugary contents of the caruncle, and in so doing help in the dispersal of the seed. The caruncle gradually diminishes as germination proceeds, but it is not possible at present to say whether this is due merely to decay or whether its substance is utilised in any way by the embryo.

If castor oil seeds are soaked and the hard testa cracked and removed, a white, slightly flattened, oval mass is seen still enclosed in a thin, skin-like inner layer of the testa. After removal of this inner skin, the surface of the mass is unbroken and gives no external indication of the parts recognised in the types so far examined. If, however, it is trimmed around its margin, it splits readily into two halves in the broader plane, and if this separation is carefully carried out, starting from the broader end of the mass, it is found that the two halves are hinged together by a small, conical radicle. The arrangement is very different from that found in the broad bean, however, for the radicle is not actually attached to the two halves of the thick mass, but to two thin cotyledons, which lie closely adpressed to the surface of the thick white tissue lying external to them. The cotyledons can be raised from this tissue, and are seen to be very thin and relatively transparent, and to contain a network of veins (Fig. 7c). With the help of a lens, it is just possible to identify a very minute plumule between the two cotyledons.

On germination the radicle emerges first and is followed by the elongating hypocotyl, which is at first strongly arched as it lifts the seed out of the soil, and then straightens out (Fig. 8a, b and c). The cracked outer seed coat is either left in the soil or carried up, whilst the cotyledons remain for some time longer partly embedded in the white tissue which enclosed them in the seed. As germination proceeds, this external white tissue rapidly becomes thinner, and is obviously being drawn upon and used up as a food supply by the germinating seedling. Finally only a thin film of it is left, which dries

* *Sarothamnus scoparius* (L.) Koch.

and breaks away as the cotyledons unfold. These are green and so leaf-like in appearance that there can be no doubt that they are of the nature of leaves, though rather different

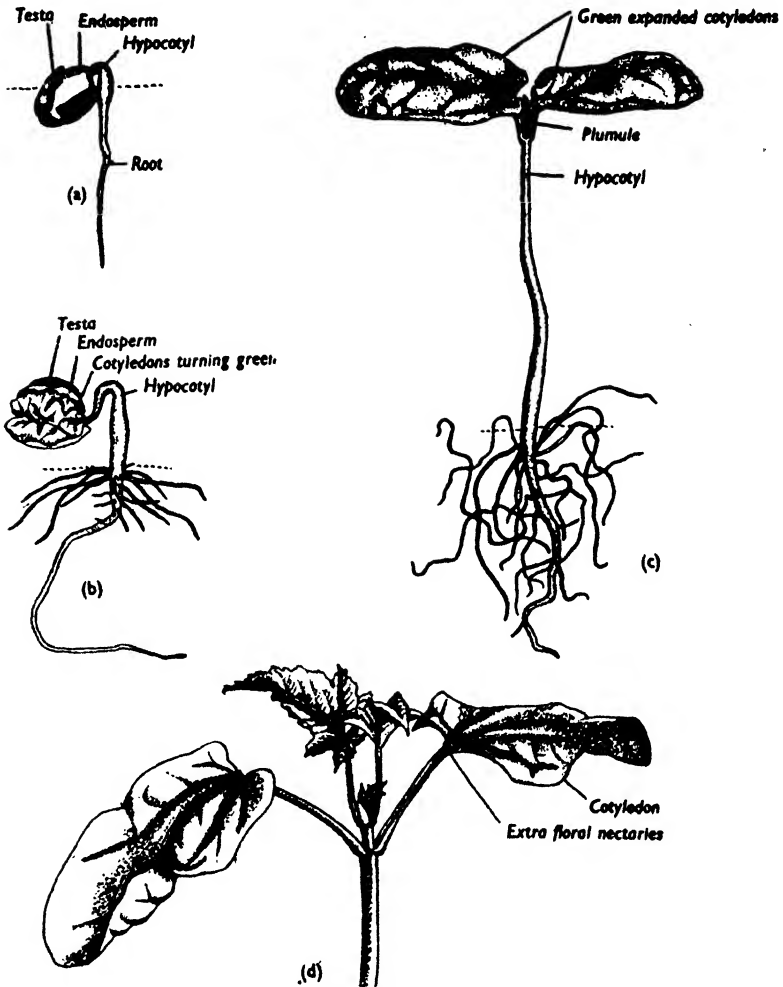


FIG. 8.—*Ricinus*, stages in germination ($\times 4$).

in shape from those which are unfolded when the small plumule eventually develops (Fig. 8d). Even in the few cases so far considered, intermediate stages of leafiness of the cotyledons have been observed, varying from those of the castor oil seedling to the thick fleshy ones of the broad bean, and comparison shows that they are all comparable structures and

so must all be regarded as leaves, although in the broad bean they have become much modified as organs of storage for reserve food. The plumule, which was seen to be so small in the seed, is very slow to show any signs of growth, and practically no difference is seen in it until the cotyledons are unfolded. In this case, little or no reserve food is stored in the cotyledons, but a food supply is present in the white mass that completely surrounds those parts of the seed which give rise to the new plant. Such an external food supply is known as endosperm. Since the cotyledons are closely adpressed to this tissue and remain enclosed in it until it is exhausted—long after the radicle has emerged—it must be through the cotyledons that this food is conveyed to the growing shoot and root. When each cotyledon is expanded, two or more small raised outgrowths are usually to be seen on the upper part of its stalk. These structures exude a sugary fluid and are similar to the nectaries which occur commonly in flowers. When found elsewhere on the plant than in flowers, they are described as extra-floral nectaries (Fig. 8*d*).

Another endospermous type is the ash (*Fraxinus excelsior* L.). The fruits are flattened and the pericarp is extended into a slightly twisted wing at the end away from the stalk (Fig. 9*a*). The fruits normally produce only one seed and do not open to liberate the seed, but if the pericarp is carefully removed from one of the flat faces, the seed is seen in position in the fruit cavity. In early stages of development the fruit of ash is found to have two cavities, in each of which two ovules (or potential seeds) are suspended by short funicles from the distal end of the cavity. As only one seed develops and fills the fruit, the partition between the two cavities is pushed to one side and appears as a long stalk to the seed, running from the floor of the fruit cavity along the narrow edge of the seed to join it at the distal end. However, if this "stalk" is examined carefully the three abortive seeds can be recognised a short distance below the attachment of the "stalk" to the functional seed and only the part of the "stalk" distal to these is the true funicle of the seed (Fig. 9*b* and *c*). The testa is relatively thin and brown, and neither the hilum nor the micropyle which lies near it are conspicuous features. The testa can be scraped off in soaked specimens, revealing the white endosperm. If this is cut round the flattened edge the seed separates into two lobes between

which the embryo is seen lying with its radicle directed towards the micropylar end (Fig. 9*d*). The embryo only extends about half-way along the endosperm, but the seed has the same general type of construction as the castor oil seed and shows the same general behaviour on germination.

From the few types of seeds so far considered, it is seen that there are two ways in which we might group them. Firstly, according to whether the reserve food is stored in the cotyledons (non-endospermic), or external to the embryo (endospermic), and secondly according to whether upon

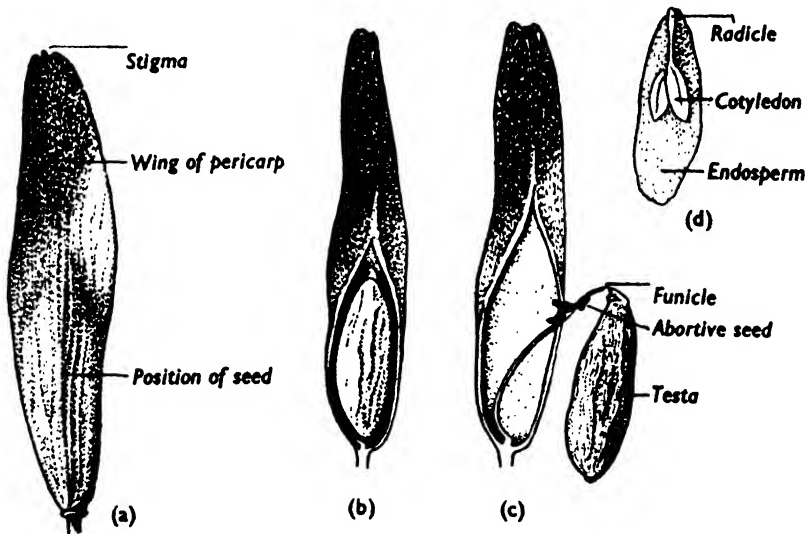


FIG. 9.—Ash, (a) single fruit, (b) with half the fruit wall removed to show the seed in position, (c) with the seed displaced to show its attachment. (d) seed split open to show embryo in endosperm ($\times 1\frac{1}{2}$).

germination the cotyledons remain below ground (hypogeous), or are carried up (epigeous). These differences prove of very different value in grouping plants, for the distinction between endospermic and non-endospermic seeds coincides with many other points of difference between the plants concerned, and is therefore of great value in classification, whilst plants with the hypogeous and epigeous seedling habits are, in some cases, very similar in other respects; and furthermore the same kind of plant, e.g. runner bean or pea, though normally hypogeous, may sometimes bring its cotyledons above the ground.

All the examples taken so far have had two cotyledons,

the presence of which characterise; the seeds of a big sub-division of angiosperms, known as dicotyledons.

The examples may be grouped as follows :—

I. SEEDS OF DICOTYLEDONS (WITH TWO COTYLEDONS).

A. *Non-endospermic*.

Broad bean (*Vicia Faba* L.) : seedling hypogeous.

Runner bean (*Phaseolus multiflorus* Willd.) : hypogeous and occasionally epigeous.

Pea (*Pisum sativum* L.) : hypogeous and occasionally epigeous.

French bean (*Phaseolus vulgaris* L.) : epigeous.

Sunflower (*Helianthus annuus* L.) (seed retained in fruit) : epigeous.

B. *Endospermic*.

Castor oil (*Ricinus communis* L.) : epigeous.

Ash (*Fraxinus excelsior* L.) (seed retained in fruit) : epigeous.

The other big sub-division of angiosperms is characterised by the presence of a single cotyledon. A similar diversity as to food storage and as to behaviour on germination is shown in this group and is illustrated in the examples now to be described.

Maize (*Zea mays* L.) is an example of the type found in cereals and grasses.

The maize grains are borne directly on a stem structure, the whole forming the well-known "cob" (Fig. 10a). As in the case of the sunflower, each grain is a one-seeded fruit, but in maize the testa is so closely fused with the fruit wall or pericarp that the two cannot be separated.

The grain is more pointed at the end where it was attached to the cob (10b); at the other broader end there is usually a depression, to the upper side of which a papilla may sometimes be recognised (Fig. 10b). This papilla bore the long, thread-like style; the styles from the numerous flowers preceding the grains form a long, silky tassel, which hangs out from amongst the sheath-like leaves wrapped round the future cob (Fig. 10a). The grains are so closely crowded on the cob that there must be considerable mutual pressure, and the ripe grains are distinctly flattened on their upper and lower flanks with reference to their position in the cob. On the upper of these flatter surfaces an area is distinguished by its opaque white appearance, the rest of the grain is more translucent (Fig. 10b). If a soaked grain is cut through vertically so as to pass through this dense region, it is found to

occupy an area at one side of the pointed end of the grain, and to be sharply delimited from the rest. Upon the addition of a drop of iodine (iodine in aqueous potassium iodide) to the cut surface, the distinction between these two areas becomes much clearer, for the small dense region only alters slightly in colour (Fig. 10c), whilst the bulk

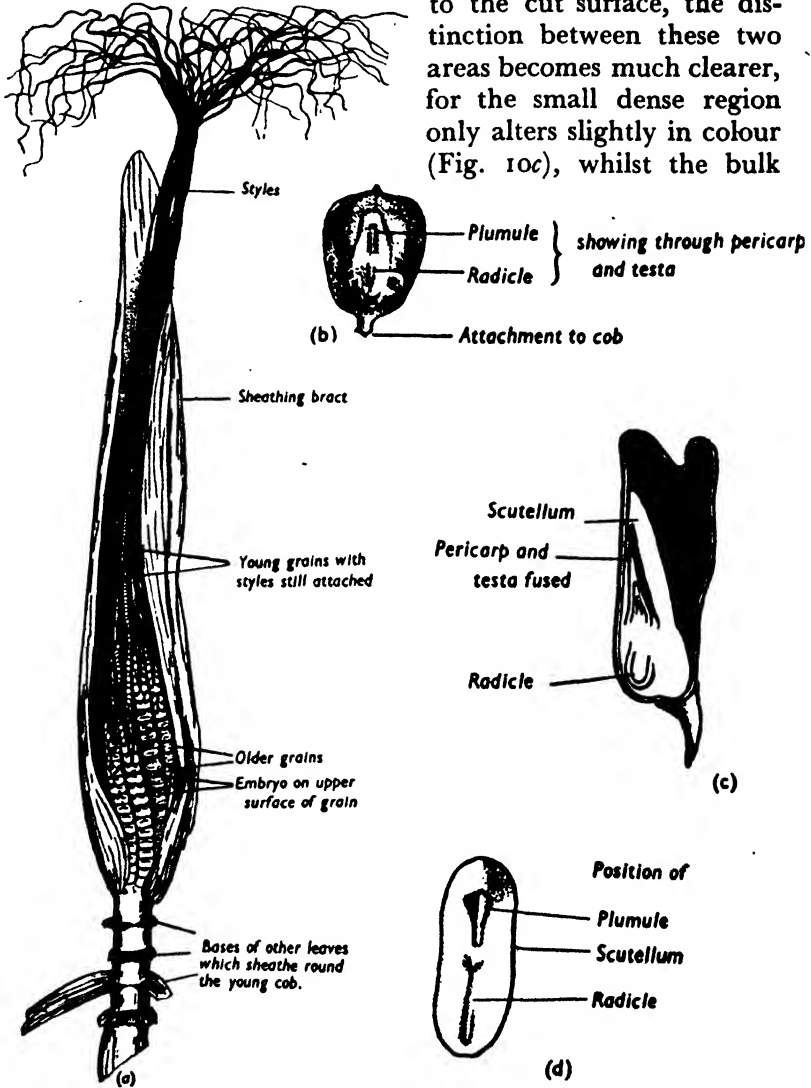


FIG. 10.—Maize, (a) young cob ($\times \frac{1}{2}$), (b) grain ($\times 1\frac{1}{2}$), (c) grain cut in half vertically and stained with iodine ($\times 2\frac{1}{2}$), (d) embryo removed from the grain ($\times 2\frac{1}{2}$).

of the grain turns black, a change in colour which indicates the presence of starch, and which consequently suggests that this is probably a region of food reserve.

With the help of a hand lens, it is seen that whilst the dark, starch-containing region shows no special structure, and is, in fact, the endosperm, the light area presents recognisable regions, which germination shows to be the parts of the embryo. In this area a conical radicle can be recognised, directed towards the pointed end of the grain, whilst the plumule, seated as usual directly above the radicle, consists of rudimentary leaf structures enclosed within a conical pointed cap. Both plumule and radicle are separated from the black, starchy region by a long, shield-like structure. Its shape can be made out more clearly if the whole opaque region is prized off from a skinned grain, when it will be seen as a wide structure, curved on the side next the endosperm and flat on the free side, where it appears to have grown completely around both plumule and radicle so as to enclose them, though only by a thin skin (Fig. 10*d*). This structure, the scutellum, is usually regarded as the single cotyledon, and examination of the bisected grain shows that it joins the axis of the embryo midway between the radicle and plumule (Fig. 10*c*). Although the maize grain is so different in construction from the previously studied types of seed, we have the same essential parts, the plumule and radicle and a food supply, which in this case is situated externally to the embryo plant, but which reaches the growing regions by absorption through the scutellum.

The parts of the embryo are small, and certainty as to their nature is only reached by watching germination. The conical radicle emerges early as the first root, and is seen to break its way through a sheath, the coleorhiza, and the tissues of the scutellum, which remain as a collar round its base (Fig. 11). The emergence of this first or primary root is followed very soon by the appearance of other roots from the same level on the embryo axis as the attachment of the scutellum. The plumule then emerges from the grain, and is at first a smooth, conical upgrowth, which may reach a length of about an inch (Fig. 11*a*). Subsequent development shows the smooth appearance to be due to an outer, conical sheathing structure which completely encloses the rest of the plumule. This is the coleoptile, and through the tip of it the first leaf tears its way (Fig. 11*b*). The young green leaves are inrolled in such a way that one lateral margin completely

encloses the other, but they rapidly unfold from the tip downwards as they develop into the long leaves typical of maize. The axis of the seedling remains very short; if, however, the

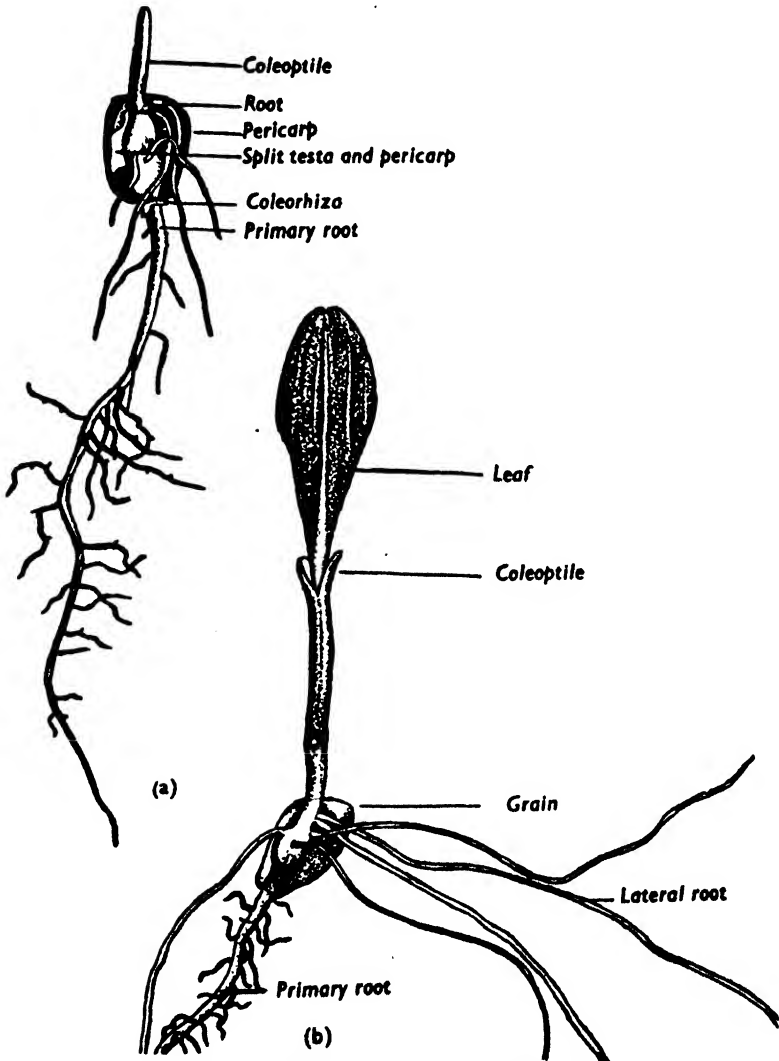


FIG. 11.—Maize, stages in germination ($\times 1$).

successive leafy structures are carefully followed down to their insertion, it is found that there is an appreciable length of axis, the mesocotyl, between the insertions of the scutellum

and the coleoptile. The length of axis separating the coleoptile from the subsequent leaves, and the latter from one another is very short indeed. The coleoptile and the leaves all completely encircle the axis at their insertion and new crops of roots tend to emerge from these various levels of attachment of the lower leaves of the shoot.

The grain containing the endosperm and the adpressed cotyledon remains below the ground, so that the seedling is hypogeous. During germination the grain becomes softer, as reserves from the endosperm are utilised for growth. Most of the reserve food is starch, but the outermost layer of endosperm contains proteins and is known as the aleurone layer.

The onion (*Allium Cepa* L.) has a small black seed liberated from a many-seeded fruit, and little of its structure can be seen, but dissection of a soaked seed shows that the black testa covers a mass of endosperm in which is embedded a small curved embryo (Fig. 12a). On testing with iodine, no starch is indicated in the tough, semi-transparent endosperm, in which the reserve is cellulose.

When the seed germinates, the root emerges first and subsequently the single cotyledon. The latter is a cylindrical, green structure which is arched at first, then gradually straightens out, carrying the testa and remains of the endosperm above the ground, whilst the tip of the cotyledon remains firmly embedded in the seed (Fig. 12b and c). The cotyledon reaches a length of 1 to 3 inches; there is a sharp contraction in diameter at its base which marks its point of insertion on the stem. Just above this point, if the seedling is held up to the light, a small dark structure may be seen inside the cotyledon. Dissection with a needle shows it to be the plumule, which is thus enclosed by the cotyledon. As the plumule develops further it emerges through a lateral, slit-like opening in the cotyledon (Fig. 12d). Evidently the tip of the cotyledon is an absorbing structure in this type since the food from the endosperm must be transferred to the embryo through it.

A type without endosperm is the water plantain (*Alisma Plantago* L.). From the flower develops a ring of numerous one-seeded fruits, flattened by their mutual compression. Each fruit is narrower on its inner face from which rises obliquely the withered style.

After preservation in alcohol the fruits are sufficiently

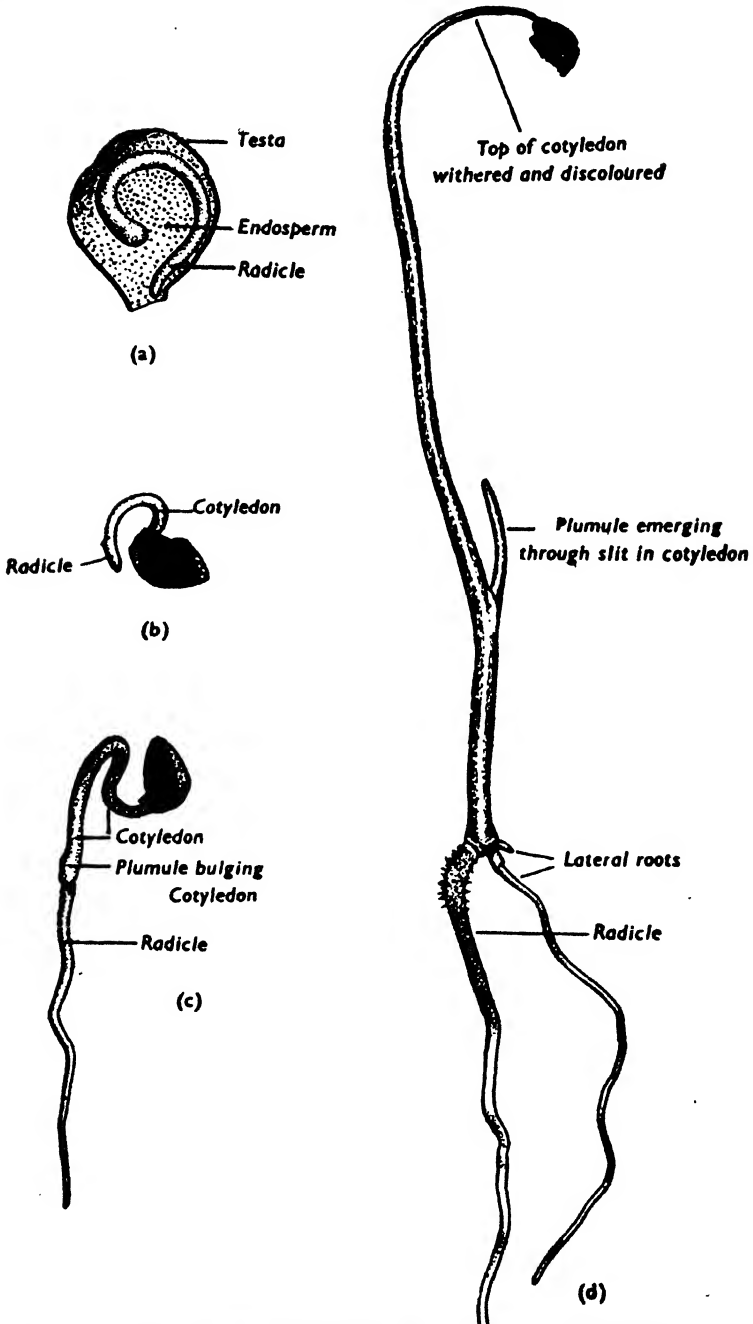


FIG. 12.—Onion, (a) seed dissected to show embryo in endosperm ($\times 9$) (b), (c) and (d) stages in germination ($\times 2$).

transparent to show the single brown seed attached to the floor of the fruit by the funicle (Fig. 13).

The seed is sharply bent upon itself so that the interior space is completely filled by an arched embryo in which radicle and cotyledon lie parallel. The thicker radicle points directly towards the micropyle, the cotyledon is narrower and more pointed. The plumule is minute and cannot be made visible without a special technique. On germination it breaks through the side of the cotyledon in the manner already described for the onion.

It is significant to find the different appearance and behaviour of the radicle and plumule in these seeds associated with the possession of a single cotyledon, and further experience confirms the impression that the presence of one or two cotyledons is indicative of differences of fundamental importance.

The examples of monocotyledon seeds which have been described may be classified as follows :—

- A. *Endospermic.*
Maize : hypogeous.
Onion : epigeous.
- B. *Non-endospermic.*
Water plantain : epigeous.

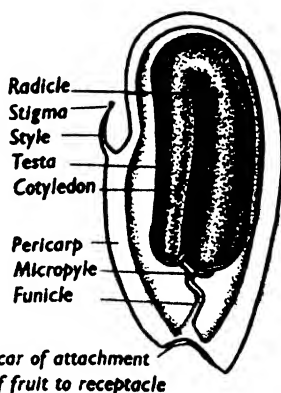


FIG. 13. — *Alisma*, single fruit made transparent to show the seed within ($\times 24$).

Practical Problems arising out of Studies of Germination.

The buying and selling of seed are important commercial transactions in the practice of agriculture. The seller is expected to be able to give some sort of guarantee as to the performance of the seed he sells—that it will give rise to plants of the same species and variety as stated at the time of sale. The fundamental problems of inheritance that are involved here will be left for consideration at a later stage (Chapter XXXVII).

In addition to a guarantee as to the specific kind of seed, it is most important to the grower, whose land is his capital and whose opportunity to earn money with it depends upon the quality of the seed he puts into it, that he should also have some guarantee as to its purity and power of germination.

If the grower adjusts the quantity of seed he plants on the assumption that 90 per cent. will grow, and actually only 20 per cent. does so, he runs the risk that his capital will not be remuneratively invested.

Seed-testing stations exist to enable the grower to give a certain guarantee of purity and germinating power at the time that the seed is put on the market. They issue, in relation to a sample, a certificate which states the percentage purity and also the percentage germination under the standardised but artificial conditions which are of necessity used. The first statement, that of percentage purity, involves relatively few problems. It is of importance that a good representative sample should be used for the test. The impurities are then expressed as a percentage by weight, and it is found in most cases that the seed on the market has a high degree of purity, usually 99 per cent., and seldom below 90 per cent., though lower percentages are occasionally met with in samples of smaller seeds such as clovers, which are more difficult to clean than larger kinds, like cereals, peas, beans, etc. Of more importance than the actual percentage is the nature of the impurity. If this merely consists of chaff, broken seeds or particles of soil, it is not serious, but sometimes the impurity includes seeds of poisonous, parasitic, or otherwise injurious weeds, or seed infected with serious fungus diseases, which it is most important to avoid. The certificate from a station, therefore, usually states the nature of the impurity.

Greater problems are involved in obtaining a percentage germination, as the whole object of the testing station is to provide a seedsmen with a certificate before the seed is sold, so that it is required as soon as practicable after harvesting. This means that an estimate as to percentage germination has to be obtained much nearer the season of harvesting than that at which the crop would normally be sown, also that the test must be carried through quickly under conditions which are entirely artificial and unlike those under which the seeds will be placed when actually sown. Nevertheless it is possible in most cases to devise tests which give some idea of the relative value of samples in this respect.

Usually sets of 100 seeds are tested for germination under standard conditions and for a standard period of time ; both of which have to be adapted for the particular kind of seed.

Seed beds of various kinds are used, but those most generally employed are sand, filter-paper or flannel, on porous plates or glass dishes, since these can easily be sterilised. These are kept in an incubator regulated to a standard temperature, and the germinated seeds are picked off at intervals and recorded. The optimum temperature and the minimum time required for a test vary with different kinds of plants. In the case of most vegetables, such as peas, and also most cereals, the healthy seeds germinate quickly and ten days is sufficient for the test, but some types, such as parsley, celery, and some grasses, require twenty-eight days, and the seeds of certain trees even longer. At the end of the time prescribed for a particular kind of seed, it is found that most of the seed which is likely to show healthy germination has already done so. The main exceptions to this general statement are types of leguminous seeds, and especially clovers, which at the end of the test period frequently show a number of apparently healthy seeds still ungerminated. If the test is continued it is found that these "hard" seeds continue to germinate at irregular intervals over a long period of time, even years. It is impossible to carry on a test to cover the period necessary for germination of all such hard seeds, so that their percentage is simply stated. In red clover samples, the percentage of hard seeds may be 2 to 3 per cent., but in English white clover it may be as high as 75 to 80 per cent., when the seed is usually subjected to some process of scarification to weaken the seed coat. Hard seed seems to owe its slow germination to extreme impermeability of the seed coat, and although a disadvantage when a uniform crop is required, hard seed may have great significance under natural conditions, for it means that germination will be extended over a long period, and if some seedlings are destroyed by a late frost, there will be other seedlings germinating later that will maintain the species.

Sometimes it is found that a uniform temperature is unsuitable. Under natural conditions in the soil, seeds must be exposed to fluctuations in temperature, and even frosts, which cause tissue expansions and contractions and very probably help to make the seed coat permeable to water. Under artificial conditions it is found that fluctuations of 5° to 10° C. improve the germination of most seeds, and in some cases (and it is interesting that these are chiefly types where the

seed is enclosed in a fruit wall as well, e.g. mangolds, beets, and some grasses), a wider fluctuation, eight hours at 20° C., and then sixteen hours at 30° C. is usually employed.

These instances, and many others which might be cited, show that considerable difficulties are encountered in obtaining a percentage germination figure for a seed sample under artificial conditions, and it has always to be borne in mind that the figures obtained are only useful for comparative purposes, and the behaviour of the same sample under field conditions might be very different.

Viability of Seeds.

Although seeds are the structures which we harvest from one crop and sow to produce a new crop, it is important to realise that a seed does not actually represent the beginning of a new individual plant, but merely a phase in its development. This particular phase stands out very clearly and is dignified by a special descriptive term—the seed—because the growth and associated changes (which are still continuing as evidenced by a slow respiration) are going on so slowly that to all external appearances the seed remains unaltered for a considerable period of time. We therefore recognise the characteristic features of this stage more definitely than is possible when growth is more rapid and more affected by slight changes in external conditions. The new plant represented in the seed actually started its development considerably earlier while it was enclosed in the fruit on the parent plant, when growth, though small in amount—since it started from a small structure, the fertilised egg (Chapter XXXIV)—was relatively rapid, and took place under conditions of ample water supply derived from the tissues of the parent. At a certain stage, usually when plumule, radicle, and cotyledons are already recognisable, the seed is cut off in some way from this original water supply. In some cases this appears to be due to the drying up and death of the plant as a whole, in others, the drying is more confined to the fruits and often causes them to open and shed the seeds; in yet others, the testa or parts of the pericarp may become so thickened and hardened that, although still on the parent plant and even enclosed in a juicy fruit, little moisture appears to penetrate to the embryo inside.

It has already been pointed out that the various chemical changes associated with growth take place in the plant in solution, and consequently this lack of moisture causes all these processes to slow down and the embryo enters upon the almost dormant stage associated with the seed. Further, since the processes are taking place so slowly, they may continue for a considerable time without appreciably altering the reserves in the seed, or causing it to become less viable, i.e. capable of germinating if placed under suitable conditions of moisture, etc.

Great variation is found in the length of time that a seed may remain viable. Broad bean seeds with 15.0 per cent. of water lose all their viability in two years, whilst turnip seeds (*Brassica* spp.) with a water content of 11.8 per cent. still have about 11 per cent. viable after five years.

As the seed is merely a stage in development, it is not surprising to find that few generalisations can be applied to it. In most cases it seems true to say that a seed is mature when it is shed, which implies that the embryo inside is already so far developed that it can at once, or after only a short period of "maturation" after shedding or harvesting, be induced to germinate if placed under suitable conditions. In this country, under natural conditions, seeds are shed during the drier summer months, and germinate with the advent of autumn and winter rain, or when warmer weather succeeds upon these wet months. In a few plants, however, the seeds will not germinate immediately after harvesting, and in some cases not until a whole year has intervened, e.g. *Anemone*, *Cratægus*. The reasons for this may be quite different and the examples given are probably cases in point. If *Anemone* fruits are examined soon after harvesting, the embryo is found to be very little developed, and appears as an oval mass without any external differentiation of parts. After separation from the parent plant, the embryo continues to develop, and if older fruits are examined they are found to contain an embryo with the usual parts recognisable. It is a case where a long period of maturation is necessary after the separation of the fruit, as the embryo is too undeveloped at that stage to germinate even under suitable external conditions. In the hawthorn (*Cratægus monogyna* Jacq.), the reason for the delay is probably to be found in the extremely hard and thick inner part of the fruit wall or pericarp, which may require a considerable period

of rotting, digestion, or mechanical injury by alternate expansions and contractions due to changes in temperature, before the embryo, which is well formed before shedding, is able to rupture it.

In some kinds of seeds the embryos are mature and ready to germinate as soon as the fruit is ripe. This is evidently the case in most of our English cereals, for if the harvest season is wet and the reaped crop not removed in time from the field for threshing, the grains germinate in the ear.

In other cases seeds occasionally germinate without a previous dormant stage. Thus in some soft, juicy fruits, such as ripe melons, tomatoes, lemons and oranges, the seeds may germinate in the fruit. Such cases are included under vivipary, a term used to denote any method in which seeds or buds begin their development into new plants whilst still upon the parent plant, from which they are subsequently shed. Particularly striking examples of vivipary are found amongst the woody plants which grow upon muddy tidal shores in the tropics. These plants are known as mangroves, but this is a general term and includes plants of different families. The seed germinates in the fruit on the parent plant, developing especially a massive hypocotyl which hangs down from the fruit with the root at its tip. The plumule is also appreciably developed but is enclosed in a sheath which holds it in the fruit until the large seedling, which may be many inches in length, eventually drops from the tree into the soft mud. Having its root well buried in the mud, the seedling has a somewhat greater chance of retaining a hold in the scour of the tide than a seed would have done.

Recognition of these facts emphasises the importance of careful drying of seed samples after harvesting, for the smaller the water content of the seeds, the longer will viability be maintained. It has been found that English red clover which is well harvested and dried shows a fall in germinating capacity of 40 per cent. in three years, whilst in a sample insufficiently dried, the same fall in germinating capacity occurs in two years.

Although the majority of seeds in nature germinate during the autumn or spring subsequent to their production, it frequently happens that some seeds are buried through the agency of cultivation, burrowing animals, or other causes, so that

when the normal time comes for their germination, they are not under suitable conditions, and if lack of sufficient air or water persists, such seeds may remain dormant for years. Tests on the period of viability of seeds of various kinds carried out under the auspices of the British Association for the Advancement of Science, showed that three or eight years were critical periods for many kinds, but as many as seven species out of 220 tested germinated after twenty-seven, and one after forty-three years. Also it was found at Rothamsted Experimental Station, that in fields which had been under grass for ten years or more, the sub-soil still contained appreciable numbers of viable seeds of arable weeds, which had evidently lain buried and dormant since the last arable crop. A striking example of retention of viability was noted during the dredging of the river Avon, near Bristol. On the mud dumps then thrown out of the river bed, species of plants appeared which had not been seen in the district for some fifty years.

The stories of germination of wheat grains from undisturbed tombs of Egyptian mummies seem to be without foundation, and the best authenticated case of long maintained viability is probably that of the fruits of the Indian Lotus (*Nelumbium speciosum* Willd.), taken from the peat at the bottom of a dried-up lake in Manchuria. From the geological evidence it seems clear that the materials of the lake bed in which these fruits are found are at least 300-400 years old, and yet the fruits show a high percentage of germination. The fruit wall or pericarp, which is present in addition to the testa, is extremely hard and so resistant to the entry of water that there is no sign of swelling after soaking in water for twenty months, and the embryo inside is still uninjured after immersing the fruits in concentrated sulphuric acid for twenty-four hours. In fact germination can only be induced by facilitating the entry of water by treatment of the coat with acid, or by mechanical injury. The conditions of preservation of these Indian Lotus fruits provide a beautiful example of longevity due to the slowing down of respiration. The fruits have highly resistant coats which do not allow water to penetrate, and at the same time will prevent the desiccation of the contents. The oxygen supply has certainly been very low, as the bed in which the fruits were found was covered with a layer of peat, the characteristic form of vegetable débris to

accumulate under conditions of low oxygen supply. The seeds enclosed in the fruits have therefore received very inadequate supplies of oxygen and water.

If the reasons for long-maintained viability of seeds are to be found in the operation of such factors as are discussed above, they may be to some extent under experimental control and of practical importance. Certain kinds of imported foreign seed, which formerly lost their power of germination in transit, are now successfully conveyed in air-tight containers. It is fully realised that seed should be stored in a cool, dry place, but with deeper understanding of the principles involved much improvement may still be made in the methods of storing and shipping seeds.

CHAPTER IV.

THE CONTINUED GROWTH OF THE SEEDLING AND THE ASSOCIATED GAIN IN DRY WEIGHT.

Gain in Dry Weight.

The average weights and dry weights of the pea plants in Table 2 show that as the plants grow older a new and very important phenomenon develops. Three weeks after sowing, the dry weight of the plant is actually less than it was originally, although the plants are now well above ground, have nearly ten times their original fresh weight, and have many leaves expanding in the light. Seven days later the dry weight of the plants has definitely increased, and from that day it continues to do so with occasional fluctuations, due to the fact that there is less uniformity amongst the plants as they grow larger, especially under field conditions (cf. Table 2, 11th August and 1st September). Evidently with continued growth new features have developed in the functional activity of the plant, and it is necessary to see if we can connect these with any characteristic features of its organisation. One point is immediately suggested by Table 4, in which weights are given for pea plants grown from the same stock of seed and under the same experimental conditions as those used to obtain the weights given in Table 3, except that in the present case they were grown in total darkness.

If the weights for the whole plants are considered, it will be seen that, whilst the fresh weight during the first eight days increased even more rapidly than when the plants were grown in the light (1.64 grams compared with 1.45 grams), yet the dry weight decreased very rapidly and subsequently continued to decrease. This result, which is always obtained in such experiments, suggests that the gain in dry weight of the normal

plant is closely associated with its growth in daylight. We must now examine the normal plant grown in the light closely to see if any feature of its development can be correlated with this transition from loss to gain in dry weight.

TABLE 4.
PEAS GROWN IN SOIL IN GREENHOUSE IN DARKNESS.
(All weights in grams.)

		Original seed.	Seedlings.			
			8 days.	20 days.	27 days.	33 days.
Shoots	Fresh weight		0.66	1.42	1.77	2.67
	Dry weight	0.0008	0.04	0.07	0.09	0.07
Roots	Fresh weight		0.31	0.39	0.29	0.33
	Dry weight	0.02	0.017	0.02	0.01	0.02
Cotyledons	Fresh weight		0.71	0.47	0.23	0.40
	Dry weight	0.21	0.13	0.03	0.017	0.01
Whole plant except testa	Fresh weight	0.26	1.64	2.40	2.45	3.51
	Dry weight	0.23	0.18	0.13	0.12	0.09
Date—1930		July 2	July 10	July 22	July 29	Aug. 6

Shoot Growth during gain in Dry Weight.

Weights of pea plants are given in Table 2, but we have seen that pea and broad bean are closely related, and exactly the same conclusions as to gain in dry weight in the light can be drawn from the broad bean. Broad beans growing in the same garden at the same time as the peas of Table 2 increased in dry weight from seed of an average weight of 1.508 grams, to give shoots on 23rd September, 1931, with an average dry weight of 53 grams. In examining the changes in form associated with this change from loss to gain in dry weight therefore, reference will be made usually to plants of the broad bean, though all points could equally well be seen in the pea. As the gain in dry weight of older plants seems to be associated with growth in light, it is the shoot that must be examined as this is the part of the plant exposed to the light.

At first the apex of the shoot resembles very much the original plumule, being still enclosed in small enveloping structures, though in the light these rapidly become green in colour. As the axis of the shoot elongates, these structures become separated from the apex and appear as the first two scale leaves of the seedling shoot. They are followed by larger leaves, which also differ in shape from the scale leaves (Fig. 14).

So many leaves continue to unfold from the apex in this way that it seems clear they were not all present on the original plumule but are being formed in succession at the shoot apex. The older and more fully-formed leaves are at the outside, and as they reach a certain stage of development they are turned out one by one from the crowded bud at the apex by the elongation of the shoot axis, thus exposing younger ones, which again in their turn enclose still younger, till the youngest of all in process of formation are in the centre of the bud and nearest to the actual apex of the shoot.

After a broad bean shoot has been growing for some time, we see that it consists of an axis, the stem, with leaves distributed along it, and it is convenient to distinguish as nodes the places where the leaves join the stem, as internodes the regions between two successive nodes. At the base of the shoot an internode separates the scale leaves from one another, and as two scales could be recognised in the seed, it is clear that this region is developed mainly by enlargement of parts already present in the plumule. The region above the scale leaves, presumably the result of the further growth of the shoot apex, consists of a quadrangular stem at each node of which is inserted a leaf, first at one angle, then, at the next node, at the opposite angle, a leaf arrangement described as alternate.

At the shoot apex the young leaves are so crowded that we cannot in this region distinguish internodes, which only become apparent when they begin to elongate and separate the leaves from one another. The elongation of the internode goes on slowly at first and then more rapidly, and usually some four internodes are elongating at the same time in broad bean.

In the broad bean it may be seen that although a leaf is well formed before it becomes separated from the apex, it continues to grow and increases considerably in size during the period of separation, and actually leaf growth and elongation of the internode below the leaf in question cease at about

the same time. The same point is illustrated by almost any leafy shoot which is still growing, but a particularly clear example is that of the garden nasturtium (*Tropæolum majus* L.), in which the increase in area of the rounded leaf blade, the elongation of the long leaf stalk and that of the internode below all synchronise and cease approximately at the same time, when they must be regarded as adult parts of the plant.

Although the leaf growth stops at a stage which coincides with the final elongation of the internode, it does not necessarily mean that no further growth proceeds in the internodal region of the plant. The internodes are often capable of increasing in girth for some time after all elongation has stopped, so that the internode at the base of an old broad bean plant is considerably thicker and tougher than it was at a time when it had just ceased to elongate. A bud, a small replica of the growing apex of the main shoot, is present in the angle between each leaf and the stem—in the axil of the leaf. Such buds may develop into branches similar to the main shoot and they are especially liable to do so when the main shoot is injured.

Although internodes may thus continue to grow in girth and buds may develop at the nodes, leaves on the other hand finally cease to grow and their associated internodes to elongate. These regions of the shoot are then spoken of as "adult" as contrasted with the growing regions. Adult and actively growing regions are both present on the plant when it begins to gain in dry weight.

In the early stages of germination, the whole seedling is growing and drawing upon the available food supply in the seed, with the result that the whole structure loses in organic matter by an amount equivalent to that used in combustion to supply the energy for these growth processes. The apical regions of the root and shoot continue to grow and to draw upon the available organic matter in the plant, but at the same time basal regions of the shoot (and incidentally, older parts of the root) emerge from the growing region and become adult. Comparison with the dry weight tables shows that this is paralleled by some compensating process, which gradually balances the first loss in dry weight, and subsequently leads to an actual gain. When a part becomes adult, it means that it will no longer utilise so much organic matter, as new parts

are not being constructed and material is used only in repairing waste, due to living processes, and to supply energy for the changes which continue after growth has ceased. In addition, adult green parts of the shoot in the light evidently have the power of making considerable quantities of organic matter; they not only increase the supplies of these substances in the plant in excess of any they themselves may use, but are obviously the source drawn upon by the growing regions. The fact that no gain in dry weight is shown by plants grown in darkness is evidence that this power of making organic matter is associated with green parts grown in light.

In both broad bean and pea seedlings the first foliar structures borne on the first nodes of the shoot have been described as scale leaves. They are certainly very different from normal leaves. Each scale leaf is relatively small and consists of two lobes with a narrow outgrowth between them. The two basal lobes or stipules are still present in the later formed leaves, but the central outgrowth is replaced by a leaf stalk or petiole bearing distally two large green lobes and a central outgrowth. Comparison of the two shows clearly that in the scale leaves only the stipules of the fully developed leaves are represented. It is not surprising to find the two types of structures very different from one another, when one considers how very different are the conditions under which they have been formed. The scale leaves have developed inside the seed, in the dark, and evidently with a deficient supply of water, and it appears that when these parts are eventually brought under more favourable conditions, they are too far matured to undergo much further expansion. The first leaves on the shoot are also developing in the seed, but at the time of germination are still sufficiently young to undergo more typical development when air and water are supplied. Both scale leaves and cotyledons are regarded as modified leaves, as they occur in the same position relative to the shoot axis and buds occur in the angle between them and the stem, as they do also in the axils of normal foliage leaves.

Plants Grown in Darkness.

Broad beans or peas grown continually in the dark are similar to normal plants at first, except that they develop no

green colour and the basal internodes are longer. Subsequent growth is, however, very different, for the plants in the dark elongate rapidly, expand no leaves, and simply carry up the shoot apex in the form of a small hooked structure (Fig. 14a). Covering the hooked apex there are a few small foliar structures, giving it an appearance very like the original plumule, and it is consequently known as the plumular hook. Seedlings grown in the dark are described as etiolated. They are very sensitive to light, but if grown with special precautions similar to those used for sensitised photographic plates and never examined except under red light, the only lateral structures expanded on the shoot are the scale leaves and even these are more adpressed to the stem and smaller than in the light. No further leaves separate from the apex, which is simply carried up by the elongation growth of the stem. If light is less strictly excluded, a few small leaves may separate from the shoot apex, but the plumular hook and excessive elongation of the internodes remain characteristic features. This growth goes on at the expense of the reserves in the seed, but after a time, during which the extreme elongation has carried the apex to a considerable distance from the food supply in the cotyledons, the main shoot collapses and dies. Supplies may then be available for other potential growing centres situated nearer the cotyledons, so the death of the main shoot is frequently followed by the development of one or more of the axillary buds, either those in the axils of the scale leaves, or more frequently those in the axils of the cotyledons. These branch shoots are similar to the original one, though more slender, and after growing for a time collapse in a similar way. The general feature of the growth of the shoot in darkness appears to be that only those structures which have reached a certain stage of development in the seed can expand, as for example, in the broad bean or pea, the two scale leaves and lower internodes associated with these scales. Other foliar organs may be present on the plumule, but are evidently too rudimentary to undergo normal growth and expansion without light and apparently, in some plants, no new foliar organs are actually formed in darkness. Another feature is that the angular stem of the normal plant is replaced by a cylindrical whitish stem, which is more like a root than a shoot in appearance. In fact one is struck at once by the colour and

general habit of the etiolated plants, in which the whitish, elongated axis, bearing small, pale yellow leaves, which never spread out their leaf blades horizontally, is very different from the normal plant grown in the light.

The comparison of normal and etiolated plants has been applied to the particular case of the broad bean, but the general facts and conclusions drawn may be applied to any type of seedling, when an interesting difference in behaviour emerges between hypogeous and epigeous seedling types.

In the broad bean, parts of the plumule which are appreciably developed in the seed undergo further development, though not necessarily to the same extent as in the light. Similarly, we find that in the case of the runner bean (*Phaseolus multiflorus* Willd.), the two leaves which were so clearly seen on the plumule, even to the details of their venation, can develop in the dark, but no further leaves are expanded, and the two basal internodes alone show extreme elongation.

Col-
lapse of the main shoot is followed by growth of buds in the axils of the cotyledons or of the basal pair of leaves.

In the seeds giving rise to epigeous seedlings a general characteristic was the extremely small development of the plumule, which in such types as the castor oil (*Ricinus communis* L.), and ash (*Fraxinus excelsior* L.), was so minute as to be practically indistinguishable without the help of a lens. In these types the plumule was extremely slow to grow in the

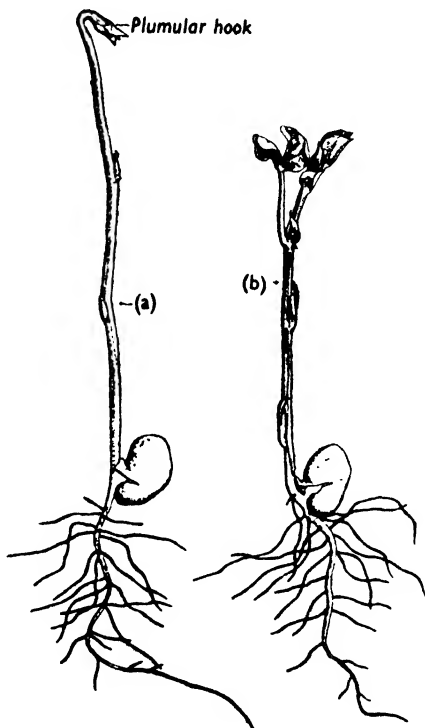


FIG. 14.—Seedlings of broad bean after twelve days' growth ($\times \frac{1}{4}$), (a) in continued darkness, (b) in daylight.

normal seedlings and showed little growth until after the cotyledons were green and fully expanded. In the dark the cotyledons are carried up by the elongation of the hypocotyl, but the small plumule is apparently insufficiently formed to make any further growth in darkness. The total growth energy of such epigeous seedlings as sunflower, castor oil, and ash, is expended in continued elongation of the hypocotyl, and no epicotyl develops. In this case also no axillary buds are present sufficiently formed to replace the growth of the main shoot, and collapse of the hypocotyl means death of the whole seedling. The cotyledons also tend to remain folded against one another and are consequently slower than normal seedlings to throw off the testa. Thus in seeds with rudimentary plumules only the hypocotyl and cotyledons grow in the dark. We have seen that the growth of an internode is usually closely associated with that of the leaf inserted above it, and in plants where the leaves of the rudimentary plumule remain undeveloped, their internodes, which would constitute the epicotyl, also remain undeveloped. In such seeds, therefore, the first growth of the seed buried in the soil must be an extension of the hypocotyl, which brings the cotyledons and plumule into the light. The plumule then develops and the extension of the epicotyl begins. Such seeds must always be epigeous in habit. On the other hand, when the plumule is well developed in the seed, as in the broad bean, runner bean, and pea, the continued development of the plumule is possible in the dark, and as the leaves of the plumule develop, the internodes of the epicotyl extend. In this type of seedling, if the growth of the epicotyl is sufficiently vigorous, so much of the available food supply may thus be drawn upon that the hypocotyl makes little growth and the cotyledons remain below ground. But the growth of either region of the axis is possible, and seedlings may be either epigeous or hypogeous according to the balance of growth between the two regions, and in the same species fluctuations in this balance may cause some seedlings to be epigeous and others hypogeous.

We might summarise the facts at present before us by saying that the gain in dry weight of a normal plant grown in the light is associated with—

(a) The formation of adult parts, since the time when gain in dry weight commences coincides with their presence,

and the gain is more rapid the higher the proportion of healthy adult to growing parts.

(b) The exposure of the plant to light, since no similar gain is registered in plants grown in the dark. Very possibly the effect of light is also correlated with the presence of green colour and expanded leaves, as these features are not exhibited in etiolated plants.

The way in which a plant is able to bring about the gain in dry weight will be considered more fully at a later stage. Now we simply observe the fact that such a gain takes place, because it accompanies the production of adult as compared with growing parts. The property of building up organic substances such as starches, sugars, proteins, etc., which constitute the greater proportion of the dry weight of a plant, from simple inorganic substances obtained from water, air, and soil, is peculiar to green parts of plants in the light. This property is most important for the continued life of the plant itself, for evidently the growing regions are not able to provide these substances for themselves and need to be supplied with them ready-made, either from reserves in the seed or from the activities of adult leaves.

CHAPTER V.

THE ADULT VEGETATIVE SHOOT.

It was evident that the original growth of shoot and root took place at the expense of the food reserves stored in the seed, and that if this drain upon food supply had not been more than balanced by other activities of the plant, the continued growth of the seedling would soon become an impossibility. Gain in dry weight in the young plant we could associate, with some confidence, with the presence of adult regions of the green plant in the light, the activities of which more than balance the loss of dry weight due to the growth of shoot and root. As a result the young plant soon has more food available for growth than had the original seedling as it emerged from its seed, and more vigorous growth is possible. In analysing this process a little further it will probably be wise to restrict ourselves for the moment to the shoot system, which is growing above ground under conditions which facilitate its study. It consists of a stem bearing as lateral members the leaves, which add interest to the inquiry by the great variety of their form, whilst the root system simply bears lateral branches similar to the main root. As a result, when trying to analyse the form of the young growing plant it is usual to say that it consists of three categories of members, leaves, stems and roots. In the present study we shall continue to treat root and shoot as the two comparable morphological categories, recognisable already as the two growing centres of the plant in the seed. A little later in this chapter we shall be in a position to examine the differences between leaf and stem and consider whether they justify their distinction as separate morphological units.


As the activities of the adult leaves supply more food to the shoot growing point, the result may be the production of

larger leaves or of more leaves of the same size, either at the same growing point or at additional growing points. An example will best illustrate the effect of this increasing vigour of growth upon the form of a plant. The following data from groundsel (*Senecio vulgaris* L.) could be paralleled from the growth of practically any seedling.

A good selection of groundsel plants of different sizes may usually be found, especially in autumn, ranging from young seedlings, showing the epigeous cotyledons still green and healthy, to old plants with numerous flower and fruit heads and also showing the lowermost leaves dying off in succession.

The following measurements were taken upon a young plant :—

PLANT A.

	Leaf.	Stem internodes.		
	Length in centimetres.	Length (cms.).	Diameter (cms.).	
	0.6	4th internode	—Not yet elongating	
	1.1	3rd „		
	2.0	2nd „		
	2.3	1st „		0.15
	Cotyledons 1.3, 1.3	Hypocotyl	0.7	0.075

In this particular case it is clear that the whole plumule is still growing, and the decreasing lengths of successive leaves merely indicates leaves at different stages of development.



In a slightly older stage the effect of increasing leaf size is apparent, and also, to a slight extent, that of increasing internodal length.

In seedling B, the lower regions are reaching their adult stage, and the effect of larger leaf size as the food supply increases is evident, even though some of the larger upper leaves have not yet ceased to grow. The internodes, always rather erratic in length in groundsel, are also a little slower in showing this effect as the internodal extension does not start until the leaves are well expanded.




Plant C shows a later stage in which the lower leaves are dying, and the main activity of the growing apex is flower production. The main axis has ceased extension growth, so

that the length of adult leaves and internodes may be taken as fully representative of the vigour of growth of the apex at the time these leaves were forming. It will be noticed that the leaves soon reach a maximum size, but that more leaves

PLANT B.

	Leaf length (cms.).	Stem internodes.					
		Length (cms.).	Diameter (cms.).				
Growing region		0.5	9th	—Not yet elongating			
		0.9	8th				
		1.9	7th				
		Increasing adult size		3.0	6th	0.1	0.175
				4.4	5th	0.4	0.175
				5.2	4th	0.5	0.175
				5.3	3rd	0.7	0.175
				3.7	2nd	0.2	0.175
				2.8	1st	0.2	0.175
				Cotyledons	1.2, 1.2	Hypocotyl	0.9



PLANT C.

	Leaf length (cms.).	Axillary branches.		Main stem internodes.			
		Length of basal internode (cms.).	Leaf length (cms.).	Length (cms.).	Diam. (cms.).		
Decreasing size associated with reproduction		} Flower branches	3.1	3.1, 2.4	10th	0.5	1.0
			4.4		9th	2.4	1.5
			5.4		8th	2.1	2.0
			6.15		7th	1.9	2.0
			6.1		6th	2.4	2.75
Maximum vegetative size		6.0	5th	1.9	2.75		
		6.4	4th	1.9	2.75		
		6.0	3rd	1.3	2.75		
Increasing adult size		Dying	3.5	2nd	0.3	2.75	
		Dead	2.5	1st	0.3	3.0	
		Cotyledons (1 dead)	1.5	Hypocotyl	0.3	0.35	

are then formed at additional growing points in the axils of these leaves. Examination will show that the smaller, lower leaves also had minute buds in their axils but these did not grow out so soon.

The length of the internode, though erratic, evidently does not continue to increase in the region where the leaf size remains constant, so that leaf length is roughly correlated with that of the associated internode, being short when leaves are small and longer when leaves are larger. On the other hand, the diameter of the internode behaves quite differently, being greater in the lower internodes. If the diameter of a basal internode be compared with that of a comparable internode of the younger seedlings, it is evident that the growth in

PLANT D.

	Leaf length (cms.).	Branch length (cms.).	Stem internodes.		
			Length (cms.).	Diameter (cms.).	
 <p>broken broken</p> <p>Leaves decayed and gone</p>	6.3				
	8.2	15	Dead in region of flowers—	5.0	0.15
	10.0	20	 <p>All flowering at apex</p> <p>Hypocotyl</p>	3.5	0.25
	11.0	22		2.5	0.4
	11.5	24		5.0	0.5
	11.5	26		3.5	0.6
	6.4	24		4.1	0.7
	11.0	28		3.5	
	11.2	30		2.9	0.7
	9.2	23		2.9	
	9.0	30		2.3	
	8.8	29		1.8	
				2.0	
				1.2	0.8
				1.8	
		0.4			
		0.7			
		0.5	0.7		

diameter of the internode does not cease with growth in length, but continues for a long period.

No such process of continued growth is evident in the leaf. On the contrary after a period the lowest leaves die. If this is still accompanied by the increasing production of new leaves of maximum size on main stem and branches, the loss of the lower adult leaves will not prevent a continued increase of dry weight, but it will be seen that upper leaves on the main shoot of D are smaller in size, and that this is associated with the appearance of flowering branches.

The behaviour of branches can be better illustrated by the measurements of another specimen, D, in which the branches are more fully grown. Vigorous branches arise from the axils of the well-grown leaves (the first leaves are already fallen in the older plant), whilst in the axils of upper, smaller leaves the branches are shorter and have produced flowers and fruit after a shorter period of leaf formation.

Measurements are supplied for the lowest branch of D also. It will be seen that the branch, unlike the seedling, forms its largest leaf and longest internode at once, whilst the reduction of leaf size and internode length further up the branch seems again associated with the transition to the flowering condition.

LOWERMOST BRANCH OF PLANT D.

Leaf length (cms.).	Basal internode.	Axillary branches	Stem internodes.	
			Length (cms.).	Diameter (cms.).
2.7		Bracts and flowers	0.8	
3.5		Bracts and flowers	1.8	0.25
3.4	1.8 cms.	One leaf and flowers	1.3	
6.5	1.7 cms.	One leaf and flowers	1.2	
6.7	1.7 cms.	One leaf and flowers	2.0	
7.0	0.9 cm.	Two leaves and flowers	3.7	0.45
8.0	0.6 cm.	Two leaves and flowers	2.9	
7.7		Bud with signs of growth	6.2	
8.5		Bud	2.0	0.35
9.1		Bud	5.8	

The data given for plant D show clearly that the growth of this plant has entered upon a new phase—a change which becomes more marked if still older plants are examined. The first indication of the altered type of growth is the falling off of leaf size and length of internode, which is then followed by the production of flowers and fruits at the various apices. During this period the older leaves are dying and no new leaves are forming at the apices, so that as the fruits mature the foliage dies away. This is followed by the drying out of the stem also, so that the whole plant dies. With this stage, clearly dry weight increase has ceased, and substance is being lost in withered vegetative parts, and in the dispersal of fruits or

seeds by which, when the individual dies, the maintenance of the species is ensured. This new phase of growth, with its difference in the behaviour of the growing points, which now produce flower parts instead of leaves, may be termed the reproductive phase. It seems clear that as this phase proceeds the gain in dry weight associated with adult leaves will gradually come to an end. The production of flowers at the growing point is a drain upon the food supply just as was the production of leaves, but when the flowers are adult they do not in their turn contribute to the dry weight and send further supplies of food to the growing points. On the contrary, they are followed by the formation of fruits, and if we consider the pea pod or bean pod it is clear that the ripening of these fruits and their contained seeds must drain away from the plant which bears them very large supplies of food, which will only become available for the next generation when the seeds germinate.

The life cycle of any groundsel plant is thus seen to pass through the following stages. In germination the seedling plant develops with the two active growing regions of shoot and root, which draw upon the original food supplies of the seed. As soon as parts of the leafy vegetative shoot become adult, supplies of food are made there which are sent to the still growing apical regions. These supplies increase in amount as the adult leafy region increases in extent, and growth becomes more vigorous until it finally changes in character and flower parts are produced in place of leaves. The development of the flowers and fruits exhausts the food supplies more quickly than they can be replenished by the decreasing leaf area, so that growth of the shoot apex ceases and gradually the shoot dies, the reproduction of the plant being provided for by the seeds which have been produced in the fruits. An examination of the form and structure of the reproductive organs is left for a later stage, but it is now necessary to examine a wider range of vegetative structures.

Leaf and Stem.

In the adult vegetative region we can usually distinguish stem and leaf, but their form and structure vary so widely in different plants that the distinctive characters tend to be

somewhat formal and are not of quite universal application. The essential feature of the aerial part of the normal flowering plant is the green vegetative shoot, through the activity of which organic matter is constructed in excess of that supplied by the seed at germination. During the vegetative phase of growth leaves are formed at the shoot apex, and when these are adult they may usually be seen to be borne in succession upon an axis. Very frequently the nodes are separated by the extension in length of internodes, which cease growth in length at approximately the same time as the leaf inserted at the next node above, though the figures for groundsel show that the internodal extension actually continues rather longer than the expansion of the next leaf above. This seems very natural in groundsel as the leaf insertion occupies only a fraction of the circumference of the stem, the remainder of which seems connected with higher leaves above, with the growth of which the extension of the internode is probably also associated.

The successive internodes comprise the stem, which is usually more or less cylindrical and very different in form from the most characteristic feature of the leaf, the flattened expanse of the lamina. Though this flattened part is usually restricted to the distal part of the leaf, it is not uncommon to find cases where it is continued down as a wing on either side of the leaf stalk or petiole, or even further vertically down the internodes below the leaf insertion. Thus, in *Helenium*, this wing-like expansion runs down from the leaf insertion through several internodes, five or six on one side of the leaf, two on the other, as a result of the way the margins of the leaves overlap one another and so interrupt the continuation of the wings. Similar lamina-like wings are well developed in sea lavender (*Limonium sinuatum* Mill.), and to a less extent in potato (*Solanum tuberosum* L.) (Fig. 20).

On the other hand, in the leaves of many plants no broad lamina is developed, as for example in the adult vegetative region of the gorse (*Ulex europaeus* L.), where it is not easy to distinguish leaves from the branch stems, most of which are of limited growth and end in sharp points. In such cases where branch and leaf are similar in form, the best criterion to distinguish the two is their relative position, since normally branch buds arise in the axils of leaves. Thus in gorse (Fig. 15a), it is possible to identify certain spiny structures as branch thorns

since they arise in the axils of other, rather flatter, sharp-pointed structures which are leaf spines. In adult gorse bushes the branch apices grow for one season only and the thorns die off during the second season; the most vigorous branches cease growth after producing a terminal cluster of leaves, the majority develop into thorns after a varying amount of growth and still others produce flowers. This being so, the continued

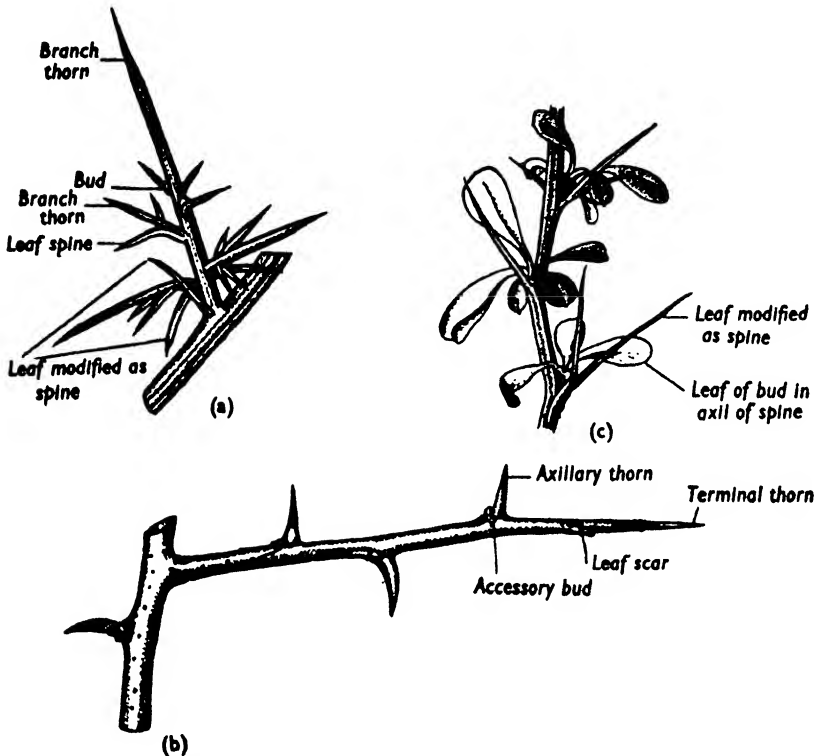


FIG. 15.—(a) Gorse with leaf spines and stem thorns ($\times 1\frac{1}{2}$), (b) hawthorn with branches modified into thorns ($\times 1$), (c) barberry with leaves modified to spines ($\times 1$).

growth of the gorse bush presents a problem, but the solution is readily seen if the bushes are observed in the spring. Each new branch appears from between the leaf of the previous season and its associated thorn and evidently arises from an additional or accessory bud, which in most cases is probably too small to grow out in the first season. These accessory buds make more vigorous growth in the second season than the main

axillary buds make in the first, and are responsible for the greater part of the increase in size of the bush.

Other good examples of thorns occur in hawthorn (*Crataegus monogyna* Jacq.) (Fig. 15*b*) and in sloe or blackthorn (*Prunus spinosa* L.). In hawthorn the amount of growth made before the apex becomes converted into a thorn point is very variable ; it is also a feature of this plant that buds in the axils of the two lowermost scales of the bud often grow out and produce short thorns or branches. In barberry the three-partite spines are equivalent to leaves and most of the expanded foliage leaves are clustered on short, axillary branches (Fig. 15*c*).

Outgrowths of a more superficial nature, known as prickles, occur on some plants on leaves and internodes in no very definite positions, as those on the rose (*Rosa* spp.), and on blackberry and raspberry (*Rubus* spp.). A peculiar example of a plant with two distinct kinds of prickles is the gooseberry (*Ribes uva-crispa* L.) ; three very characteristic spines are formed around the leaf insertion, one at either side and one on the back of the leaf base ; smaller prickles with no very definite distribution also appear along the internodes. The comparatively superficial nature of the prickles in rose is shown by the ease with which they may be separated from the stem, no vein being broken in the process.

The position of the bud in the axil of the leaf also facilitates the distinction of a sub-divided or compound leaf from a stem with numerous small leaves. Thus the numerous leaflets of a large leaf of ash (*Fraxinus excelsior* L.) or elderberry (*Sambucus nigra* L.) might be mistaken for simple leaves on a shoot such as privet (*Ligustrum vulgare* L.), but the presence of a bud only where the large compound leaf joins the stem and in the axil of each simple leaf in privet would distinguish the two. Of course no mistake would be possible in such cases if development were followed, when the compound leaf would be seen to unfold from the apex as a whole, whilst the pairs of leaves in privet would expand in succession. Even the position of the bud, however, will not always provide the distinction between stem and leaf. Many leaves have no bud in the axil, as, for example, the majority of the leaves on a conifer such as spruce (*Picea Abies* Karsten), nor is any bud found where a *Cyclamen* leaf joins the swollen stem, whilst in *Tolmiea Menziesii*. Torr. et Gray, buds are found at the

junction of leaf blade and petiole, as well as in the axil of the leaf.

The leaf is, in fact, like its associated internode, a part of the vigorous outburst of growth of the vegetative shoot apex. Usually distinct in form and structure it is also, in the dicotyledon particularly, characterised by a short vegetative life, and when it dies back, the internode, which ceased to grow in length at about the same time but has continued to grow in girth, remains alive, and often, as the leaf withers, forms a very clean scar across the region where the leaf joins the stem. This leaf scar, a cleanly healed surface of separation, is found on the stem, and nothing equivalent can usually be found at the base of the fallen leaf. It is another indication that, at the time of separation, growth was still proceeding in the tissues of the stem and not in the leaf. After its growth has ceased a leaf may persist on the stem for a long time, even through several growing seasons, as in the evergreens such as cherry laurel (*Prunus Laurocerasus* L.), holly (*Ilex Aquifolium* L.); in one gymnosperm often grown in this country, the puzzle monkey (*Araucaria imbricata* Pav.), the leaves may persist for more than twenty-five years. In all cases, however, the leaf has expanded fully in its first growing season, and no increase in size normally takes place after this. In one plant however, *Streptocarpus polyanthus* Hook., vegetative growth is maintained by the persistent growth of one of the original two cotyledons alone, until the reproductive phase is reached, when a growing point arises from the base of the petiole of the cotyledon and gives rise to flowering stems, then leafy shoots may also appear.

Whilst most flowering plants then, behave as groundsel and at the zenith of their vegetative activity have produced a number of green leaves of about the same size, exceptions may be found, but all flowering plants that are capable of obtaining their food supplies from soil, air, and water by the aid of light, will prove to have a phase of shoot growth which is associated with the production of an adult green surface, which may or may not fall within our definition of leaf and stem; they will then usually begin to enter a reproductive phase which is associated with the production of flower and fruit.

CHAPTER VI.

THE LIFE CYCLE OF THE INDIVIDUAL PLANT.

THE flowering plant, as it grows from seed, will always pass through a vigorous vegetative phase during which it gains in dry weight, to a phase of flower and fruit production in which the dry weight is used up in seed production. This last phase, however, may not end, as is usual in groundsel, in the death of the individual ; if some growth centres continue to form leaves, or if new growth centres recommence the formation of leaves, the shoot may again gain in organic matter and resume its growth activity. In this way the life of the individual plant may be sustained for a very long, even an indefinite period, so that one may distinguish amongst flowering plants between monocarpic plants that die after the first reproductive phase, and polycarpic plants which survive through many such phases. In our English climate the frost of winter may accelerate the death of the individual, so that there is a tendency to distinguish between annual plants that die within the year, and perennial plants that persist over several years, but the more fundamental distinction is between monocarpic and polycarpic plants.

In Table 5 the dry weights of fruit and seed are compared for shepherd's purse (*Capsella Bursa-pastoris* Medik), which is a monocarpic plant like groundsel, and for the polycarpic rose bay willow herb (*Epilobium angustifolium* L.).* The plants were gathered when the shoots were withering and the fruits ripening to discharge the seeds. The table indicates the type of result to be obtained. These figures suggest that a relatively greater proportion of the dry weight of the shepherd's purse has been utilised in the production of flower and seed.

In the case of the willow herb the abortive fruits and flower buds formed late in the season are not included in the weight

* *Chamenerion angustifolium* (L.) Scop.

LIFE CYCLE OF THE INDIVIDUAL PLANT 69

of either fruits or shoots, and it is difficult to know how much of the underground shoot and root system to include, but none the less it is clear that a much smaller proportion of the total dry weight is being expended in the production of flower and

TABLE 5.
DISTRIBUTION OF DRY WEIGHT IN GRAMS BETWEEN VEGETATIVE AND REPRODUCTIVE REGIONS.

<i>Capsella Bursa-pastoris</i> Medik.	Dry weight.	Percentage of total dry weight.
Plant A.		
Carried 180 full pods *	0·504	38·7
Seeds *	0·324	24·8
Rest of plant	0·8	61·3
Total dry weight	1·304	
Plant B.		
Carried 1276 full pods *	3·57	44·8
Seeds *	2·29	28·7
Rest of plant	4·4	55·2
Total dry weight	7·97	
* Each pod averaged about 28 seeds.		
<i>Epilobium angustifolium</i> L.		
Plant A.		
76 large fruits, 91 small, 127 buds		
76 large fruits †	3·07	9·3
Seeds of these †	2·17	6·3
Rest of plant except root	25·7	77·5
4 inches of root pulled up	4·4	13·2
Plant B.		
40 large fruits, 28 small, 50 buds		
40 large fruits †	1·62	7·1
Seeds of these †	1·14	5·1
Rest of plant except root	19·4	85·7
3 inches of root pulled up	1·63	7·2
† Each large fruit averaged about 420 seeds.		

fruit than in the shepherd's purse. It is therefore not surprising to find that when the upper flowering and fruiting regions wither, as the fruits dehisce and the seeds are discharged, growth is still found to be going on in the lower regions of the

plant. It will be remembered that in the seedling groundsel the buds in the axils of the lower smaller leaves were minute and did not grow out into branches like those in the axils of larger leaves higher on the plant. The small buds at the base of a seedling *Epilobium* behave similarly at first, but later in the season, when proportionately less food is being utilised in the production of fruit and seed, these buds develop further. They do not grow out immediately into elongated branches, but produce a number of pairs of relatively rigid leaves, almost scale-like in character. When the main flowering stem dies down, these large buds, thus produced at soil level, remain dormant through the winter and grow up into erect leafy and flowering shoots the following spring. On this shoot the scale-like leaves of the winter bud make little further growth and are associated with short internodes, whilst the region of the shoot with larger leaves and longer internodes is due to new growth in the spring, so that, although produced from a bud, this shoot has certain features in common with a seedling shoot. Analysis of the shoot shows the same features as in groundsel, an increase in leaf length and internode length to a maximum size followed by a decrease as the reproductive phase is entered, and again buds in the axils of the largest leaves grow out into branches. In this plant the leaves are borne in pairs in the vegetative part, but occur singly at the nodes in the reproductive region. In shepherd's purse the plant dies down naturally before the onset of winter frosts, because the food is so completely drained away to the fruits that the lower buds do not grow out to produce fresh leaves; the plant is a natural annual and the life cycle is completed in the English summer. Other short-lived monocarpic plants, such as chickweed (*Stellaria media* Vill.) and annual meadow grass (*Poa annua* L.), die down in the same way after vigorous seed production, but many of the seeds shed may germinate the same summer, and more than one crop may be completed in the course of the season. Such plants are sometimes distinguished as ephemerals, and amongst their number are some of the most prolific weeds of the garden. Groundsel (*Senecio vulgaris* L.) is also a monocarpic plant, but the life cycle often tends to be even longer than that of the shepherd's purse, so that whilst the earlier plants are exhausted by reproduction, upper parts of the shoots of the later ones are killed by frost before the food supplies

are completely exhausted, and the lower buds, in more sheltered positions near the soil, may grow out and survive for a time.

The cultivated biennials are also monocarpic plants, but the life cycle extends over two growing seasons. Some of these plants in their uncultivated form tend to behave as annuals, others as perennials. In the cases where the cultivated races have been derived from annuals, individuals with exceptionally long periods of vegetative growth have been selected and used as parents for the production of seed. In this way races have been developed which differ from the original type in that most of them, under the conditions of their cultivation, reach the height of their vegetative activity at the end of the first season. The food materials produced during this period are then stored in the plant over the winter and used in the following season in the formation of an erect flowering shoot and a crop of seeds.

The food may be stored in various parts of the plant body, which undergoes exceptional development in the process.

Swollen roots : Carrot (*Daucus Carota* L.).

Parsnip (*Pastinaca sativa* L.).

Swollen hypocotyl : Turnip (*Brassica campestris* L.).

Swollen hypocotyl and base of leafy stem :

Swede (*Brassica campestris* L.)

Radish (*Raphanus sativus* L.).

Swollen root and hypocotyl : Beet (*Beta vulgaris* L.).

Swollen stem above ground : Kohl-rabi (*Brassica oleracea* L. var. *caulo-rapa*).

Swollen main bud : Cabbage (*Brassica oleracea* L. var. *capitata*).

Swollen axillary buds : Brussels sprout (*Brassica oleracea* L. var. *bullata gemmifera*).

Swollen young inflorescence : Broccoli (*Brassica oleracea* L. var. *botrytis asparagoides*).

Cauliflower (*Brassica oleracea* L. var. *botrytis cauliflora*).

Under cultivation the biennials are gathered at the end of the first season, when they have accumulated the maximum amount of organic matter. The biennials are, however, such specialised, selected races of plants that with variations in the season, or with a different strain of seed, the maximum of vegetative vigour may no longer coincide with the end of the first season of growth, and the plant may begin to develop flowers and to transfer the food materials towards the centres

of seed production before the winter. Such aberrant plants are most striking in a field of cultivated biennials, for the stem usually remains comparatively short during the vegetative phase of growth, but shows considerable elongation when the flowering shoots develop. In a warm, wet season, and with a susceptible strain of sugar beet or swede, it is possible to see fields in which 40 to 50 per cent. of the crop is tending to flower at the close of the first season. Such early flowering specimens are known as "bolters," and provide a very serious problem in the cultivation of such crops. From our present point of view, however, such plants only vary from the general habit of their kind in that they tend to flower earlier. All these plants are monocarpic, for the production of seeds exhausts the supplies in the plant so completely that death of the individual ensues. There is obviously no reason why the life cycle of a plant, which dies upon flowering, should be completed within a year, provided that all seasons of the year permit of continued growth or, at least, survival. In the English climate, those plants which cannot complete their life cycle within the one year, and are not able to survive the winter frosts, are eliminated from the natural vegetation, but in tropical or sub-tropical regions the vegetative phase of a plant may continue for longer periods. In such regions there seems no reason why the life cycle of a monocarpic plant should not extend over many years before it is terminated by the production of flowers and fruit. A curious fact, however, is that all well-known examples of such long life cycles appear to be monocotyledons. Thus the *Agave* may grow vegetatively for many years—20 to 100 in the case of a plant known as the American aloe under greenhouse conditions. A large branch system of flowers is then produced, and the ultimate production of fruit and seeds so exhausts the plant that death of the individual follows. Most of the species of aloe are but rarely branched in the vegetative region, and the same is true of the Talipot palms (*Corypha umbraculifera* L.), which have the same habit of flowering and dying after a period of about 100 years. The Bamboos (*Bambusa* spp.) branch repeatedly from the base like so many other members of the grass family, so that after growing in this way for some years the same plant is represented by a dense clump of branches. When the flowering time arrives, the apices of all the various branches

give rise to the feathery flower masses and subsequently the whole clump dies down simultaneously. The life cycle in the bamboos of S. Brazil is about thirteen years, whilst *Bambusa arundinacea* Willd. in India has been recorded to have a cycle of thirty-two years. The result may be most striking as extensive areas of country are often covered by bamboos developed from seedlings of the same age, derived from the fruits of one clump which previously flowered in the vicinity. The new clumps consequently tend to flower at the same season, and the aspect of large tracts of country may be transformed as the green hues of the vegetative clumps give way to the pale feathery plumes of the flowering regions, and then finally to the brown hues of the dying plants. Although the clumps of bamboo may represent individual plants, it is possible to split such clumps into smaller parts, and in this way parts of the same individual plant may be distributed to other countries, and it is interesting to find that the period of the life cycle is frequently not appreciably altered by the different conditions under which they may then be grown. It has been recorded on occasions that the supplies of bamboos which have reached this country from the same district and subsequently been distributed to various botanic gardens have all flowered and died simultaneously, so that this particular kind of bamboo has been lost under cultivation.

It is clear in the light of these examples that the distinction which is fundamental is not whether a plant lives for one year or many years, but rather whether it is monocarpic or polycarpic. This distinction again must not be pressed too far. The death of the monocarpic plant is due to the excessive movement of food to the growing points producing flowers and fruits. If we prevent this drain of food away from the basal regions where dormant basal buds are always present, these buds will grow out and produce more leaves before flowering, and thus the vegetative life of the plant may be prolonged. In this way it is possible to maintain an ephemeral like the chickweed as a perennial under greenhouse conditions by simply nipping off the young flowers as they appear. Similarly in nature, conditions may so modify the balance between flower and leaf production that a plant, normally monocarpic, may not be exhausted in flower and fruit production and thus the individual may persist beyond its normal span.

The Individual and the "Clone."

So far we have considered mainly the life cycle of the shoot in monocarpic plants. A much wider range of shoot types is likely to be found in polycarpic plants in which the almost unlimited growth of the individual provides opportunity for so much fuller a display of the potentialities of the growth system of the shoot.

That all flowering plants might be prolonged indefinitely as individual plants simply by restricting flower production, or that many kinds of plants have achieved naturally such a balance of reproductive and vegetative phases of growth that no limit in terms of years can be set to the normal life of the individual plant, has many consequences which are not readily grasped by minds more familiar with the limited span of human life.

At the outset arises the problem that it is frequently difficult to determine the limits of the individual plant. For example, many plants, such as the creeping buttercup (*Ranunculus repens* L.), develop horizontal branches near the soil level, which root at some distance from the original plant and produce leafy shoots bearing branches, that in turn repeat the same performance. For a time such branches may remain in connection, but usually the connections die away after a time, as does also the original plant, and the rooted branches appear as isolated individuals, though they are all as much parts of the original plant as if the connecting links were still present.

Consideration of the shoot system of polycarpic plants provides many instances of ways in which plants may be multiplied by removing parts and growing them as separate entities. Such methods of multiplying parts of an individual plant are described as vegetative propagation, as contrasted with reproduction by seed, in which the individual plants of the same species are multiplied. The fundamental difference between these two methods will become more apparent when the details of development of the seed from the flower are examined. Seed production usually results from the coming together or fertilisation of a part of the flower of one plant by pollen from the same or another plant. The seedlings derived as a result of such acts of fertilisation are different from the

plant on which they are produced and cannot be considered as integral parts of it, whilst the branches taking part in vegetative propagation are continuations of the identical tissues which built up the original plant. An important result of these facts is that individual plants derived from the seeds off the same parent plant may differ from one another as much as individuals of the same human family, whilst even widely separated parts of the same individual tend, so far as incidences of climate, soil, etc., will allow, to grow into plants with characteristics as similar as if they were still branches side by side on the same plant.

It is obvious that when we come to deal with individuals that can be split up into a number of pieces, each of which can grow into a plant, we are using the term "individual" in a sense that was not contemplated when the term was introduced in relation to human individuals, and a new term—the "clone"—is therefore coming into use to cover these multiplied growing fragments of one original plant. Such "clones" are often the "varieties" of horticultural practice. Thus one of the best-known varieties of strawberry is that sold under the name of Royal Sovereign. The plants spread by means of horizontal branches, and so long as new plants are obtained only from rooted branches of this kind, the quality of the fruit remains uniform, and can be recognised as that of the Royal Sovereign type. But if the "seeds" from a strawberry of this kind are used as the starting point of a new crop, the resulting fruits may differ in many respects from the standard type and cannot be sold under this name.

The potato is another outstanding example of a plant which is put on the market under a number of horticultural names, each referring to a different "clone". Problems in relation to this plant are discussed in more detail in Chapter VII.

CHAPTER VII.

HERBACEOUS AND WOODY PERENNIALS.

ALL polycarpic plants are necessarily perennial, but they fall into various categories. In the English climate it is probable that some of the plants which behave as annuals, are forced to do so by their susceptibility to frost. The different degree of resistance to frost shown by plants of different kinds is little understood, but the susceptibility appears to be greater in plants or parts of plants which contain relatively high percentages of water. The seedling shoot and any young growing regions always have a high water content, but one of the changes that follows upon the attainment of the adult stage is a gradual rise in the relative proportion of dry to fresh weight. The older the internode the longer this type of change has been going on, and it is very common to find the lower and older internodes resistant to frost whilst the younger parts die back. This difference may also be due to some extent to the fact that the older internodes may be protected from cold, dry winds by their proximity to the soil. In most plants of the dicotyledon and conifer groups, these changes in the older internodes are associated with growth in girth, when the difference in the appearance of the younger watery parts of the shoot and the relatively dry, woody, older parts, may be very striking. Such changes may proceed to very different extents, and in many plants the stem is only very slightly different in appearance by the end of the growing season, and that it is still susceptible to frost is shown by the death of the exposed aerial parts. Such plants are grouped as herbaceous perennials, for the stem remains predominantly green whilst alive, and the plants persist from one season to another by survival of parts near or below the surface of the soil. In other cases the changes may be more marked and also proceed so rapidly that the basal parts have become brown and woody

before the first frosts, and in normal seasons such stems can withstand the frost and drying effect of the wind. Growth commences from the buds borne on these woody branch systems in successive seasons, and we have the possibility of much more extensive types of plant body. Such plants provide another category, the woody perennials.

We must now consider some of the more characteristic growth forms of the herbaceous and woody perennials. Woody perennials are chiefly dicotyledons and conifers, but herbaceous perennials are either dicotyledons or monocotyledons. These two latter groups have, however, such different characteristics of shoot growth that it seems wise to give separate consideration to these two types of herbaceous polycarpic perennials.

Herbaceous Polycarpic Perennials.

I. DICOTYLEDONS.

Our experience of shoot morphology has been limited to what may be called a "typical" shoot, growing erect with elongated internodes. The length of internode usually increases up to the period of maximum vegetative activity, and then falls off again in the reproductive region. It has also been assumed that the axillary buds are similar in nature to the shoot apex, and that, if induced to develop, they will grow out into branches similar to the main shoot. If the short, basal internodes, bearing dormant vegetative buds, are sufficiently resistant to survive the winter, the plant becomes a perennial, for in the following season the dormant buds may give rise to a clump of new branches. As a similar type of growth is continued year after year the size of the clump increases, unless artificially divided. If such a plant is dug up in winter the most obvious feature is a tangle of roots, among which are the persistent bases of branches which have flowered and then died down, and on these the living buds are easily recognised. The persistent part of this type of perennial is known as a root-stock, and plants perennating in this way are the main constituents of the herbaceous border, e.g. michaelmas daisy (*Aster* spp.), perennial cornflowers (*Centaurea* spp.). The herbaceous plant shoot, however, may take many different forms, in fact these are so numerous that it is only possible to review a few of the most characteristic.

The Rosette Type.

In many plants the internodes are not elongated between successive leaf insertions, so that the plant appears as a rosette of leaves produced close to the ground on an extremely short stem or root-stock. Though this is the usual habit of growth, elongated stems with scattered leaves can be induced to develop in many plants of the rosette habit by artificial treatment and are frequently formed naturally at certain stages of development.

Examples of rosette plants are not lacking among monocarpic annuals, e.g. shepherd's purse (*Capsella Bursa-pastoris* Medik.), and this crowded rosette of leaves is a characteristic of most of the biennials in their first year of growth; but this type of plant is best represented by the polycarpic plants of our rockeries, most of which are perennials and natives of alpine regions, e.g. stonecrop (*Sedum* spp.), saxifrage (*Saxifraga* spp.), house leek (*Sempervivum tectorum* L.). In the annuals and biennials of this type, as well as in the majority of the perennials, the plant is a typical rosette up to about the maximum vegetative period, but with the falling off of leaf size prior to flowering, the internodes begin to elongate, and the inflorescence with its reduced leaves is usually carried well up above the original rosette of leaves.

In a perennial plant of this habit, such as house leek (*Sempervivum* spp.), below the rosette of green leaves there is a length of brown stem on which the leaves have been separated by some internodal elongation. From some of the buds on this stem, or from axillary buds at the base of the rosette, new branches arise with elongated internodes at first, but which produce a new rosette as soon as the apex of the branch emerges into the light. The probability that such elongated branches are the result of growth under conditions of insufficient light is supported by the fact that the house-leek may be induced to develop elongated internodes by growing it in darkness. The new rosettes usually develop close around the parent rosette, but if a branch grows between rocks it may continue its elongated growth for some distance before emerging into the light and producing a new rosette. Decay of the basal parts of the branches or artificial separation of the rosettes brings about the multiplication of the original plant, and such branches are known as offsets.

We are as yet very ignorant as to the factors at work in the production of this type of growth habit which results in the suppression of internodes, but it is obvious that plants of this kind will have enhanced chances of survival in exposed, windy situations, as the young growing regions cling so close to the ground that they are often spoken of as "geophilous" or "earth-loving." They form the characteristic type of plant in alpine and sub-arctic regions and also of exposed semi-desert regions such as many parts of South Africa. Such plants are protected from the drying effect of wind, and so are able to survive in habitats where most plants with erect shoots would be unable to live. The green clusters of leaves persist for years, and when the first shoot eventually dies, the new rosettes carry on an independent existence.

Horizontal Stems.

We have assumed that the branches on an erect shoot will behave like the main shoot and also grow erect. This statement, however, requires some qualification, for clearly the branches, being lateral, must grow at an angle to the main stem. In a normal branching shoot the upper branches grow almost vertically, but lower down the shoot system the branches approach nearer and nearer to the horizontal. The lowermost branches often arise relatively late from small basal buds which were practically dormant in early stages of the shoot's growth, and such branches show a great tendency to differ from the normal branches. Frequently they grow out into long "runners" rooting at the nodes.

The creeping buttercup (*Ranunculus repens* L.) is a good example of a plant that spreads by runners. The main shoot is of the rosette type and only elongates appreciably in the region of the inflorescence (the name given to the part of the shoot bearing the flowers). The buds in the axils of the lower leaves grow out into runners with long internodes and with a leaf at each node. The buds in the axils of the leaves on these runners develop into new rosettes and a crop of roots grows out from the bud axis and from the node, so that should the long internode be broken the effect is merely to separate the rooted branches from the parent plant. By this means the plant spreads with great rapidity in all directions, especially when it is growing in a damp habitat.

The violet (*Viola odorata* L.) also spreads by runners; these, however, bear scale leaves, and normal leaves are only produced by the rosette-like plants which develop from the axillary buds on the runner when it has grown beyond the shelter of the leaves of the parent plant.

The propagation of clones of the strawberry (*Fragaria vesca* L.) depends upon its habit of producing runners. As in violet, the leaves produced directly on the runner are reduced (Fig. 16). The normal leaves of the strawberry have a

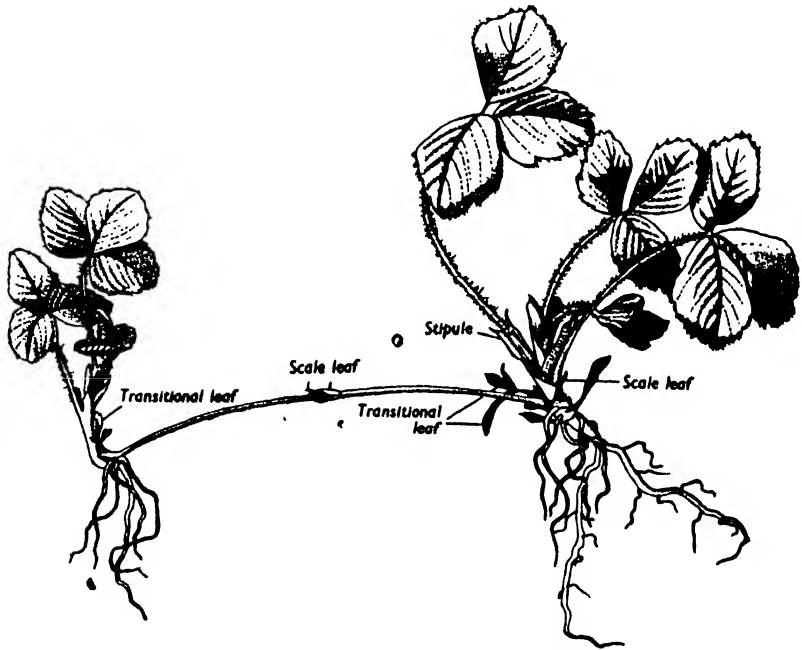


FIG. 16.—Strawberry runner ($\times \frac{1}{2}$).

stipule fused to the leaf base at either side and the scale leaves are three-cleft in shape; the central tooth represents all that has developed of the main region of the leaf, and the lateral teeth correspond to the stipules. The first scale leaf on the runner has an axillary bud, but this bud seldom develops. When the runner is in the light it may bear a normal leaf with stipules, petiole and three-lobed lamina, or else a leaf type intermediate between this and the scale leaf. The terminal bud of the runner now produces a new rosette plant with a new crop of roots. The continuation of the runner beyond this point is

by the outgrowth of a bud in the axil of a lower leaf of the new rosette and not as the continued growth of the growing point of the same runner as in the case of the creeping buttercup.

In some plants the horizontal stems from the lower axillary buds become much more prominent than the original erect shoot. Thus in bugle (*Ajuga reptans* L.), the main seedling shoot grows erect and eventually flowers, but the branches are all horizontal and greatly exceed the main shoot in length (Fig. 17). In ground ivy (*Glechoma hederacea* L.), even the main shoot of the seedling bends over and grows horizontally. Both this and the lateral branches root periodically and the apex of either type may at certain times turn up into the vertical direction and produce an inflorescence. In many

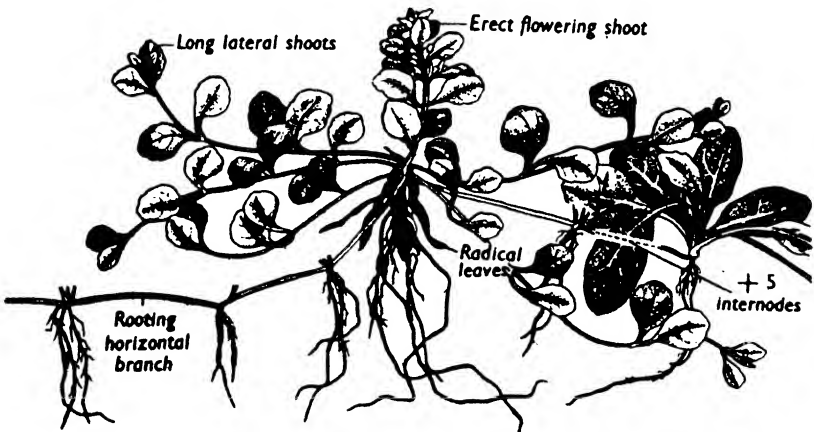


FIG. 17.—Bugle, showing horizontal branches from lower buds ($\times \frac{1}{2}$).

such procumbent weeds with all the stems creeping, as in field speedwell (*Veronica agrestis* L.), the original seedling shoot cannot be recognised in adult plants.

The basal buds which give rise to the runners of violet and strawberry grow out close to the soil and under the shade of the rosette of leaves of the parent plant. In many plants the basal part of the stem becomes buried in soil, so that the buds grow out as etiolated shoots. They often follow a more or less horizontal course, but after growing for some distance in this way, the apex reaches the surface of the soil and grows into a normal leafy shoot. Such underground stems are described as stolons if they are slender, or rhizomes if thicker, but the two terms are not used with any great consistency. Coltsfoot

(*Tussilago Farfara* L.) is a good example of a plant which spreads in this way. Buds which develop in the light produce a rosette of leaves each with a long petiole and a large blade; those below ground develop into stolons bearing small, white scale leaves separated by internodes often as much as two inches in length (Fig. 18). A stolon may extend a considerable distance before the apex emerges into the light and produces a new rosette. When a vigorous bud reaches the light the terminal bud forms the crop of foliage leaves; the buds in the axils of lower foliage leaves or scale leaves just beneath the rosette are re-

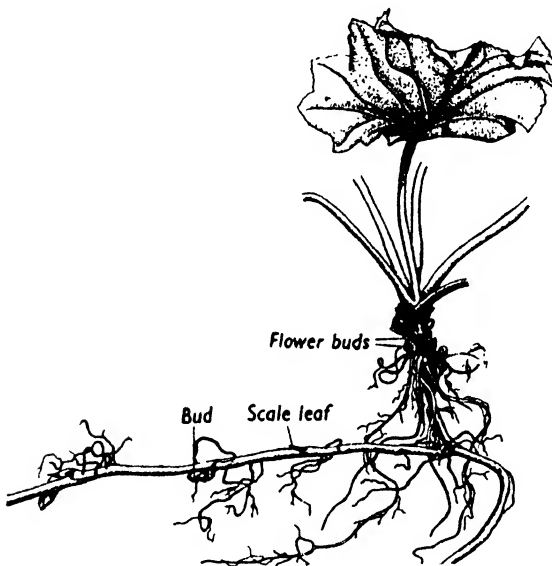


FIG. 18.—Coltsfoot, vegetative condition ($\times \frac{1}{2}$).

productive buds, whilst the buds in the axils of scale leaves deeper in the soil grow into stolons. At the end of the season the leaves die down, leaving the inflorescence buds buried just below the surface of the soil. Early the following spring these buds expand, each producing a stem with numerous scale leaves upon it and a terminal

head of small, yellow flowers (Fig. 19). The apical bud in the centre of the rosette usually dies with the foliage, though it may develop into a terminal inflorescence bud, in either case the growth must be continued the next season from lower axillary buds.

In enchanter's nightshade (*Circæa lutetiana* L.), or dog's mercury (*Mercurialis perennis* L.), the plant spreads by similar means. On the erect shoots of either of these plants, the leaves are inserted in pairs as are also the reduced scale-like leaves upon the stolons. Rather thicker underground stems, better described as rhizomes, occur in the field thistle (*Cnicus arvensis*

Hoffm.)*, and make it a particularly troublesome weed to eradicate.

The common perennial sunflower of the garden (*Helianthus decapetalus* var. *multiflorus* A. Gray) bears a cluster of short stolons, each of which ends in a slightly swollen region just behind the terminal bud and on which the scale leaves are borne in pairs. Another species of this same genus is the Jerusalem artichoke (*Helianthus tuberosus* L.), in which the stolons are again short but the swollen tuberous region of the bud is more pronounced. These two types are good examples to illustrate the transition from the stoloniferous to the tuberous habit.

Numerous other cases of underground stems might be cited, but the tuber-bearing stolons of the potato (*Solanum tuberosum* L.) deserve special mention. In this plant the erect aerial part of the shoot consists of a stem, winged along the most conspicuous angles (Fig. 20). The green leaves are compound with

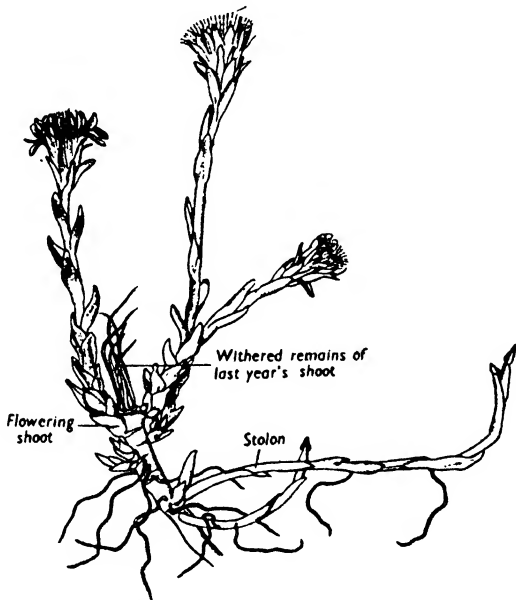


FIG. 19.—Coltsfoot, flowering condition ($\times \frac{1}{2}$).

a terminal leaflet and several pairs of lateral leaflets joined by a wing along the leaf axis or rachis. The leaf always has a bud in its axil and in aerial parts these may grow out into typical branches. If the lower internodes of the stem are earthed up, the buds in the axils of the lower leaves grow out into underground stolons. These differ from the aerial stems in their white colour, in the absence of wings along their internodes and in the reduction of their leaves to small scales. The ends of these stolons ultimately swell up into rounded or oval tubers. The tuber is thus

* *Cirsium arvense* (L.) Scop.

simply the swollen distal portion of the stolon and on its surface the spirally arranged scale leaves with their axillary buds or "eyes" (Fig. 21) can still be recognised. At the apex of the tuber, or "rose end," the leaf scales follow one another more closely, and the "eyes" appear more crowded

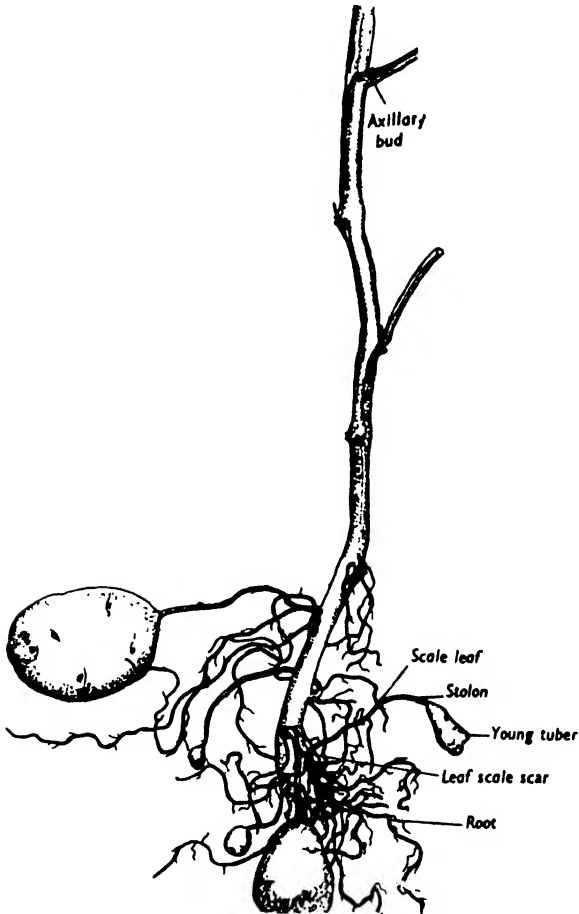


FIG. 20.—Base of potato plant with tubers ($\times \frac{1}{4}$).

than at the basal, or "heel" end. Much starchy reserve food is stored up in these tubers when the maximum size is reached.

If the base of the shoot has been well earthed up, a number of such tuber-bearing stolons may grow out around the parent stem. These are readily separated from the parent and offer

a means of vegetatively propagating the individual and so multiplying the clone. The potato is perhaps the outstanding example of indefinite multiplication of an individual plant, since potatoes, in horticultural practice, are always grown from tubers. It is possible to raise potatoes from the seeds which are occasionally produced in the yellow-green, tomato-like fruits, but for the first few years the tubers on seedling plants are too small and few in number to be of commercial value. Furthermore, the plants produced from seed may have properties very different from those of the parent plant, and in such ways new forms are obtained. If a potato is required

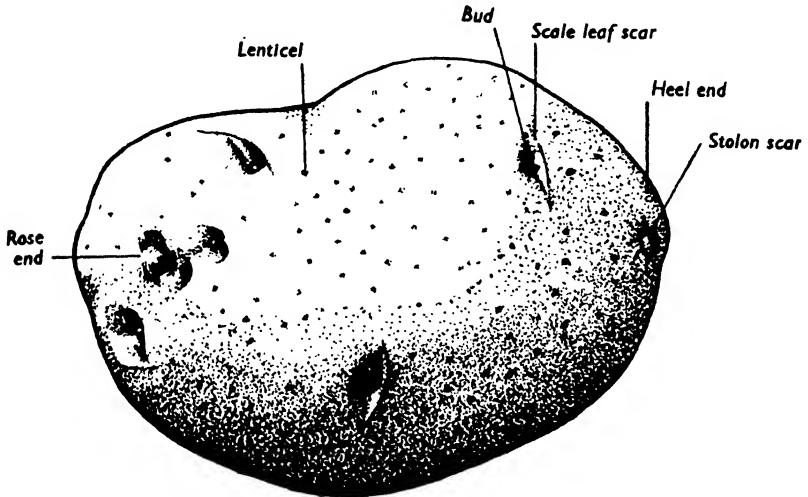


FIG. 21.—Potato tuber ($\times 1$).

with particular qualities, such as those associated with, for example, the King Edward potato, the only method to ensure their production is to grow the new crop from King Edward tubers, and so by actual vegetative continuity carry on through new seasons the original King Edward plant, which would originally have been a seedling especially selected from amongst other seedlings for these qualities. It is important to realise that all King Edward potatoes are thus parts of one plant and members of a clone, whilst Arran Comrade potatoes are all parts of another plant and collectively another clone. It is in relation to potatoes that the question of the length of the life of such a multiplied individual plant has been chiefly discussed.

Looking through a grower's catalogue to-day, it is noticed that the named "varieties," more strictly the clones, of potatoes advertised, are different from those in the catalogues of some years back. This is partly due to the selection of new and improved types, partly to the fact that when a clone has been grown for some years, it appears to become less vigorous and to produce smaller and less satisfactory crops of tubers, whilst the tops often appear unhealthy. This deterioration was previously regarded as due to the effect of the increasing age of the individual now widely spread as a clone. Recent work has shown, however, that potatoes are largely contaminated by "virus diseases," which are so-called because no visible causal organism has so far been identified. These diseases cause blotching, curling, and other abnormalities of the leaves, which prevent their normal functioning, either in the building up of organic matter or in its transference from the leaves to the tubers. If a clone becomes infected with a virus, the effects appear to be cumulative and may become so serious that a clone may disappear from the market, to be superseded by others more recently derived from seed. That the deterioration is due to virus has been shown in the case of Champion potatoes—a clone which went off the market some years ago owing to deterioration. It has been possible to retrieve an uninfected plant from this clone, and it was then shown that in spite of its age, Champion potatoes could be grown which were as good as the original.

The production of tuber-bearing stolons bears a definite relationship to the growth of the aerial parts. Although possibly a native of semi-tropical latitude, the potato as a commercial crop is essentially a plant of more temperate regions, as only under rather colder climatic conditions is there a marked tendency for stolons to be produced. In tropical countries the top growth is so long continued that most of the organic matter manufactured in the adult leaves is used in the growth of further erect branches and there is little or no development of stolons. In very cold conditions, on the other hand, the top development may be very slight or altogether suppressed, and the tuber may only form underground shoots. In such cases the food stored in the original tuber is simply transferred to a new crop of tubers, and since no new organic matter has been formed in green leaves

expanded in the light, the new set of tubers are small. This may take place in the field in a cold spring, when apparently the planted tuber has failed to grow, but is found to have given rise to a whole crop of small tubers. This fact is made use of sometimes to obtain new potatoes out of season. The tubers are kept over the summer and then planted in a cool, dry place, when the substance of the old tuber is transferred into a crop of new ones.

That food is being transferred from the top or potato "haulm" to the underground parts is illustrated by cases of injury or fungus attack to the base of the green shoot. Under such conditions the food cannot move below ground, and goes instead into the axillary buds in the axils of the leaves above the injury. These swell up into small tubers that differ from the normal ones in their green colour and the fact that they are not formed at the end of stolons, because stolons are only formed in darkness and these tubers are formed from buds in the light.

The crop of potatoes is, as would be expected, heavier if the plant does not flower and fruit. In most of the herbaceous polycarpic perennials, we have seen that flowering does not altogether deplete the plant of organic food materials, but sufficient remains in the basal or underground part to maintain alive certain buds, which grow out into new shoots the following season. In the plants belonging to this class, which are especially selected as crop plants, flowering is often eliminated, and all the material which normally goes to seed production is stored instead in vegetative parts, which carry on the plant from year to year and vegetatively propagate the clone. In such structures the vital living parts of the perennating regions are the buds, but these are small structures and contain little reserve food in themselves, so that an important accompaniment to the buds is the root-stock, stolon or tuber in which food supplies are present. In most of the cases so far considered the storage organ has been of the nature of a stem, and the buds have been in the axils of leaves, though these are often very small in the etiolated, underground forms.

Certain cases of biennials have been cited (p. 71), in which the organic matter is stored in the root and utilised the following season in the production of the flowering shoots. Among perennials there are also examples; in the root-stocks of the garden dahlia or the water dropwort (*Ceanothe*

crocata L.) some of the roots become swollen into storage organs or root tubers. The dandelion (*Taraxacum vulgare* Schrank) and many plants of the dock family (Polygonaceæ) also perennate by means of roots, and in one example of the latter family, the sheep's sorrel (*Rumex Acetosella* L.), the plant spreads by underground, brown, elongated structures, which at first sight resemble stolons. Careful examination, however, shows that no leaves are borne on these structures, and though branch roots are borne either singly or often in clusters along their length, such clusters show no definite spacing as was the case when roots emerged from the nodes of stolons. These nodeless structures are roots, as can be verified by their anatomical characters. Buds frequently arise from these roots, usually from a part close to the emergence of a branch root. These buds develop into new shoots, and it is often difficult to decide whether the first leaf is on the branch structure or on the root, though careful examination always shows it to be inserted actually on the bud axis. The unequal spacing of these buds and absence of any indication of leaves or nodes are the more obvious distinctions between these roots and stolons. The production of buds on roots is not infrequent. It may be demonstrated very readily by planting pieces of the root of sea-kale (*Crambe maritima* L.) in damp sand, when buds are freely produced from the cut surface.

CHAPTER VIII.

HERBACEOUS POLYCARPIC PERENNIALS.

II. MONOCOTYLEDONS.

The Monocotyledon Habit.

The range of form exhibited by monocotyledons is so closely associated with certain features of growth characteristic of this group that it is helpful to have these clearly in mind before considering the perennial polycarpic forms.

In the dissection of the maize grain it was seen that the presence of only one cotyledon was associated with a type of leaf insertion distinct from that seen in the dicotyledons. The cotyledon and the succeeding leaves in maize, and also in onion, completely encircled the axis at their point of insertion and consequently only one leaf could be borne at any one level. This point is so fundamental that to it may be traced the distinction upon which the two large sub-divisions of the flowering plants have received their names. In the embryo the shoot axis usually remains too short for leaves above one another to reach appreciable development, but in the dicotyledons two develop at almost the same level and become the two cotyledons, whilst in the monocotyledons, the wide leaf insertion may account for the fact that only one seed leaf is present.

Dissection of rather older maize seedlings shows that the first leaf to break through the coleoptile, and all the later leaves, show certain characteristic parts. At the base, the leaf completely surrounds the stem and from this point extends up for some distance as a cylindrical sheath with overlapping margins. The blade which is developed from the top of the sheath is borne at a slight angle to the latter, whilst at the point of junction and continuing the same line of direction

as the sheath is a small scale-like upgrowth, the ligule. The blade, as it grows in width, becomes inrolled at the margins, but this inrolling disappears as the leaf subsequently emerges above the older leaves and expands. When some of these inner leaves, which are adult at the tip, where they may even be turning brown and dying, are followed downwards during the dissection it is found that the texture changes to a yellow and delicate tissue which is obviously still growing. Evidently the leaf continues to grow for a long time and the most active region of growth is the basal region of the blade; the long narrow shape of the grass leaf and of many other monocotyledons seems to be associated with this basal mode of growth.

As the leaves are removed from the maize seedling it is seen that the basal internodes are short, but in older plants the successive leaves increase rapidly in size whilst their associated internodes become longer, until leaf size, internodal length and diameter attain a maximum, which remains more or less constant until a decrease in vegetative vigour is induced by the transition to the reproductive phase. The first increase up to a maximum exactly parallels the behaviour of dicotyledonous seedlings, such as groundsel, but with the interesting difference that, whereas in the latter type the base of the plant after it had ceased to grow in length continued to grow in thickness, in the monocotyledons, grown from seed, it is a general character that the axis at the base retains the narrow diameter of the seedling (Fig. 104). This is due to the absence of any continued growth in girth after elongation has ceased: such continued girth growth only occurs extremely rarely in monocotyledons.

The manner of leaf growth may be examined in the common spider-wort of the greenhouse (*Tradescantia fluminensis* Vell.). The adult leaves consist of a cylindrical closed sheath about a quarter of an inch long, which narrows off to a neck-like region before widening out into the broader lamina. The lamina is broadest in its lower half, and tapers off distally to a point. The plant creeps over the soil or other support and the leaves are borne in two ranks. The sheath forms a closed cylinder in which the line of junction of the two free margins of the blade is marked by a vertical line of hairs, which is also continued down the internode below the leaf insertion, a case comparable with the development of wings down a dicotyledonous internode as continuations of the margins of the next

leaf above, e.g. *Helenium*. This fact suggests that here, as in the dicotyledon, we may expect a close connection between the development of a leaf and the internode below. This is borne out by the dissection of the growing region (Table 6).

TABLE 6.
GROWING TRADESCANTIA SHOOT.
(Lengths measured in cm.)

	Internode.	Leaf sheath.	Leaf lamina.	
9	—	—	0.6	Margins inrolled except for 0.5 cm. from apex.
8	—	0.05	2.3	Margins inrolled except for 1.4 cm. from apex.
7	0.1	0.2	4.1	Margins inrolled except for 2.6 cm. from apex.
6	0.5	0.6	5.1	
5	1.7	0.65	5.6	
4	3.7	0.7	5.5	
3	5.4	0.6	5.7	
2	5.2	0.6	6.1	
1	4.8	0.6	6.6	

The series measured show that growth in length of internode, leaf sheath, and lamina occur at the same time, though their periods of maximum growth and time of cessation do not exactly coincide. In leaf 7 the lower half of the lamina is still growing, as shown by the inrolling of the margins, its yellower colour, more delicate texture, and the fact that if unrolled the leaf is seen to be narrower in the lower half than an adult leaf. In the same leaf the sheath is only $\frac{1}{3}$ grown and the internode has only just started to elongate. By leaf 5 the lamina and sheath are full grown, but the internode is only about $\frac{1}{3}$ its final length, which is not attained until below leaf 3. The growing internodes become tough and adult at the top first, and continue to grow longest at the base where they are enclosed by the sheath of the leaf inserted at the node below. The bud is found in the axil of the leaf sheath on the same side as the blade. Roots often occur at the nodes, breaking out on the side towards the soil; but these do not make their appearance until the leaf and internode above have ceased to grow. Once the internodes are fully extended they undergo

tyledon stem must mean that food is continually reaching this region, even when all vigorous adult leaves are borne at higher levels on the stem. If food can descend so far it may descend further and thus the continued growth of the main (tap) root below the base of the stem is provided for. It is probably not an accidental coincidence, therefore, that in monocotyledons where the basal internode soon ceases all growth, there is usually no permanent tap root below this internode, but new roots develop successively from higher and higher nodes. Thus the root system of the monocotyledons forms what is termed a fibrous system as contrasted with the tap root system of the dicotyledon.

Branching in Monocotyledons.

The degree of branching in monocotyledons is very variable; in monocarpic aloes or in the talipot palms the stem remains practically unbranched, whilst richly branched forms are well illustrated in the grasses. In the grasses the behaviour of the buds as they develop into branches gives rise to plants of very different habits. In annual meadow grass (*Poa annua* L.) the bud in the axil of almost every leaf develops into a branch, which, however, grows up inside the sheath of the subtending leaf, adpressed to the main shoot. Since the internodes in the vegetative parts remain short, this means that all the branches arise close together and the result is the production of a plant of closely tufted habit. This particular plant is monocarpic, for all the branches pass over into the reproductive phase and the plant dies down as it becomes exhausted with seed production. The tufted grass habit is also seen in many of the polycarpic grasses, such as tussocks (*Deschampsia cespitosa* Beauv.), or cock's-foot (*Dactylis glomerata* L.), for in these types all the branches do not reach the reproductive phase in the same season, and those which remain vegetative survive the winter and continue growth the next spring.

In some of the other perennial grasses a straggling habit is produced because the branches tend to grow out at an angle from the main stem and the growing buds either break out through the base of the sheath of the subtending leaf or push it away from the stem. In the light such lateral branches remain short and simply produce a tuft of leaves, but if they grow out very near or below the soil level, they develop into

stolons, rooted at the nodes, and with elongated internodes and small scale leaves which are equivalent to the sheath only of the normal grass leaf. When the growing apex of a stolon reaches the light it develops into the normal type of shoot with short internodes and bearing leaves with sheath and lamina. Grasses of this habit cover large areas of ground and consequently some of them, such as bent (*Agrostis alba* L. var. *stolonifera*)*, may be useful in the composition of a turf; some, on the other hand, are among the most troublesome of weeds on arable land, as couch grass (*Agropyron repens* Beauv.), for the rooting stolons bear buds in the axils of the scale leaves, so that almost any portion of a stolon may serve as a rapid means of vegetative multiplication.

Methods of Perennation.

In perennial grasses the plants continue to bear some green leaves during the winter season, but growth is very slow during this period and is only resumed again actively in the spring. In many monocotyledons the vegetative organs die down at the end of the growing season, and food made by the green leaves in the light is sent down and stored in some special region of the plant to serve as a supply to the buds when they resume growth again after a dormant period. The perennating parts of the plant in which this food is stored become much swollen, and it will be found that storage always takes place in those regions which are still capable of growing to accommodate the food stores which are being passed into them. In monocotyledons the regions which will still be capable of growth relatively late in the season are the bases of the long leaves, the internodes associated with these leaves and of course the buds.

An instance of food storage in the internodes associated with green leaves may be illustrated by the rhizome of iris (*Iris germanica* L.) (Fig. 22). The rhizome is a thick structure about an inch in diameter, which runs horizontally near the surface of the soil. The rhizome may go on growing year after year, its length being added to by the same apical growing region which passes through a cycle of growth each successive season. The story of its past behaviour is then illustrated in the shape of the rhizome, which consists of a series of swollen joints connected to one another by narrower regions and of

* *Agrostis stolonifera* L.

which each joint represents the growth of one season. Exactly as in the shoots of maize, the growth of iris rises to a maximum and then falls off in autumn as conditions become less favourable for growth, and the outline of the rhizome might be taken as the graph indicating vigour of vegetative growth. The perennating organ in this case is a stem with very short crowded internodes, each of which thickens up as the food from the leaf inserted at the next node above is passed down into the stem, and naturally the internodes associated with the

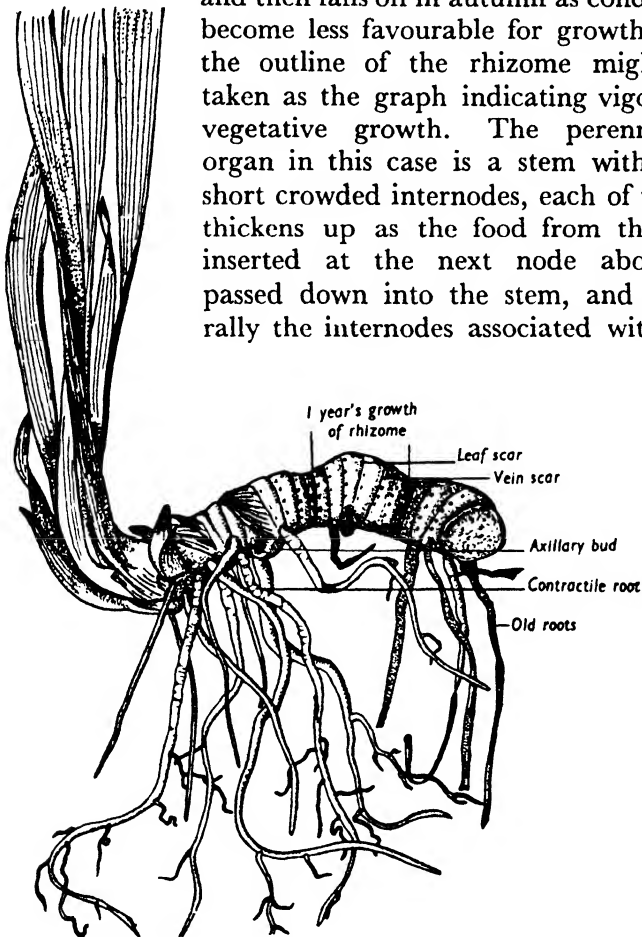


FIG. 22.—*Iris* rhizome ($\times \frac{1}{2}$).

largest leaves are the largest and have the greatest amount of food stored in them. Each growing season the new portion of the shoot expanded from the bud consists of a cycle of leaves and a new crop of roots from the lower side of the stem. The remains of the roots of former seasons persist on older parts of the rhizome, but it is obvious that it is only the more resistant outer parts of these which have not rotted away. Leaves are only present on the current season's growth, and if these

are dissected off it is found that they range from the oldest which are brown and dead, through others which are adult, or adult at the tip though still growing at the base, to the younger ones around the centre of the growing bud. The leaves are inserted alternately on either side of the rhizome and the insertion of each completely encircles the node, but the sheath is split to the base at one side and here also the scar left by the leaf is narrower. An axillary bud is present on the side where the sheath is intact. This arrangement of alternating wide scars (with buds subtended) and narrow scars can be followed back on the flanks of the older part of the rhizome. Occasionally lateral buds grow out into branches, but more often the rhizome remains unbranched so long as it continues to grow vegetatively.

The leafy shoot of the iris is much flattened and the leaf is sharply folded along the median line. A little higher the two inner faces are fused together and the leaf is continued as a sword-like blade in which there is no exposed upper or adaxial surface.

When an iris rhizome has been growing vegetatively for some time the apex may pass over into the reproductive phase and the terminal bud, after bearing leaves, gives rise to the erect branched axis of the inflorescence. This axis dies back to the rhizome after flowering, and a scar is left in the position of the former terminal bud. The next year the growth of the rhizome is carried on by the growth of one or more of the axillary buds, so that the rhizome then appears to have changed its line of direction or to have forked. Year after year the leafy portion of the iris plant is thus produced an inch or so farther away. Although no new leaves or roots are produced on older parts of the rhizome, these still contain a quantity of reserve food, and for some years it is possible that occasional buds, which have remained dormant, may grow out. Eventually the older parts die away and the branches become separated as distinct individuals. By this manner of growth irises planted in one place in a flower bed will gradually spread out into a larger circle, but it is also noticed that irises, like many other monocotyledons, tend to move particularly towards positions where conditions for growth may be more favourable. When the roots on a rhizome in autumn are examined, it is commonly seen that the older ones on the current year's growth are

wrinkled at the base ; this is actually due to a contraction of the root as it gradually dies and the wrinkling effect is to be traced to the fact that the inner tissues contract more than the less elastic outer zone. The distal regions of the root system may bear numerous young branches in close contact with the soil, so that when the root system contracts on dying the position of the rhizome alters rather than that of the distal, much-branched root system. Thus if plants with this habit have made very asymmetric root growth, when soil conditions are much better on one side than another, the subsequent death of the root system, and associated contraction by a percentage of its total length, will pull the rhizome of the plant towards the side with longer roots and therefore towards the more favourable soil. These contractile roots are very characteristic of monocotyledons, and they explain to a considerable extent the gradual displacement of such plants in the soil. In *Sauromatum guttatum* Shott., a species allied to the arums, the roots may contract to about half their original length and thus draw the stem down 6 inches in the soil. Similarly, if a rhizome be buried too deeply the roots will grow from it upwards through the soil, and then subsequent contraction will carry the rhizome upwards towards its normal growth level.

In Solomon's seal (*Polygonatum multiflorum* All.) the white rhizomes grow deeper in the soil than those of iris and probably for this reason have roots arising all over them ; these roots also remain alive on relatively old parts of the plant. The plant owes its English name to the regular series of inflorescence scars which are seen along the upper surface of the rhizome, for this plant normally flowers every year. Each autumn a large white bud is present, enclosed in a series of white scale leaves, and this will continue growth the next year. About six of these scales can be dissected off the bud, when they are seen to leave narrow scars on a short stem, which even at this stage is already swollen (Fig. 23). Each year a portion of rhizome is formed with about six scars on it, so that evidently in this case the region where food storage takes place is the internodal region associated with the scale leaves. When the scales have been dissected from the bud, a short length of stem, bare of foliar structures, is exposed, and this is terminated by a bud with a series of young leaves so regularly arranged in two opposite vertical rows as to give a plaited appearance. On dissection

these prove to be foliage leaves, in the axils of which small groups of flower buds are present. The inner portion of the bud, after removal of the scale leaves, is of a yellow colour, quite distinct from the white of the scale leaf region. This yellow

region rises above the soil in early summer and bears the green foliage leaves with axillary clusters of white flowers. The whole of this aerial shoot subsequently dies down, so that evidently the food manufactured by the green leaves in the light is passed down and stored in the

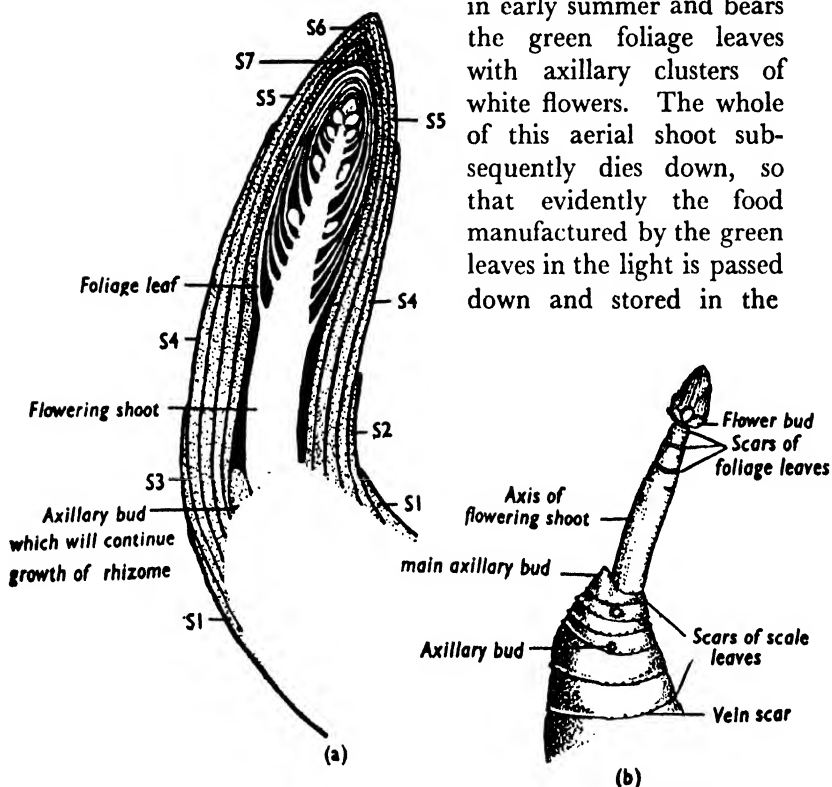


FIG. 23.—Solomon's seal, dissections of bud, (a) bud wrapped in scale leaves S_1 to S_7 , cut longitudinally ($\times 3$), (b) bud with all scales and six foliage leaves removed ($\times 2$).

internodes between the scales of the bud which will carry on the growth of the rhizome the next season. This bud is situated in the axil of the highest scale leaf of the shoot (Fig. 23).

In the corms of crocus (*Crocus vernus* All.)* we have another case in which the internodes of the stem are swollen up with the reserves of food stored in them. This very short erect axis looks very different from a typical rhizome as the older parts do

* *Crocus albiflorus* Schult.

not persist so long, so that the main portion of the corm is really equivalent to one "joint" of the rhizome of iris.

The crocus corm is a thick rounded disc, capped by one or more buds; in autumn it is enclosed in a brown fibrous covering and usually has a ring of roots around the lower end (Fig. 24). The fibrous cover consists of several overlapping layers, each of which leaves a circular scar on the swollen stem. A bud is found in the axil of each, often a little above the actual line of the scar. The arrangement of the buds shows that the leaves were inserted in a three-ranked series. At the top of the corm there is a depression, on the sides of which the ring scars are more closely spaced and sometimes subtend larger buds. At the base of the depression

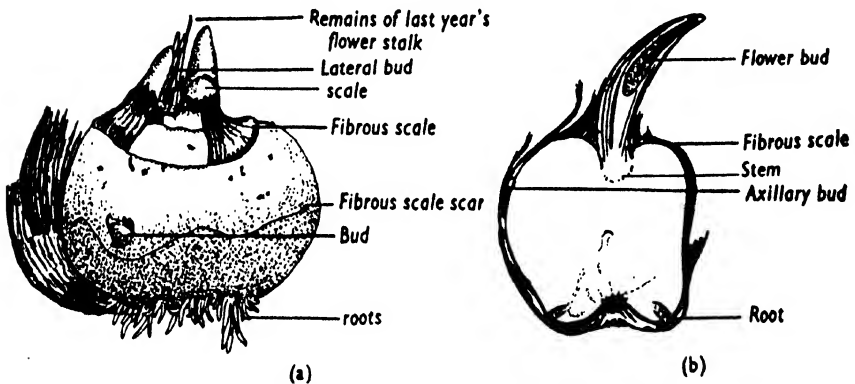


FIG. 24.—Crocus corm, (a) entire, (b) in median section ($\times 1$).

are inserted one or more large elongated buds, enclosed in white scale leaves. In the centre at the top of a large corm will be found the brown remnant of the old flower stalk. Dissection of one of the large buds shows the usual cycle of structures within; an outer series of about six encircling scale leaves, in the axils of which it may be possible to see the small buds, several foliage leaves of which the different nature is clear by their yellow colour, narrow blade, and very short, encircling sheath, and finally a few young flowers, each enclosed in a sheathing scale. As these structures are removed it is seen that already in autumn the axis of the bud is beginning to swell up to form the next corm, and that at this time the region which is swelling consists of the internodes between the scale leaves.

If crocus corms are dug up when they are just at the end of their flowering season, it will be seen that the original corm has become much shrivelled by the withdrawal of food from it to supply the buds as they grew out to form the leaves and flowers of the current season. When the green leaves have been expanded in the light they make more food and this is passed back to be stored in the internodes to form the corms of the next generation, and one or more of these swelling corms will be superposed on the old one. If these are dissected it will be found that the axis between the scale leaves of the bud, though now thicker, remain very short, and it is the internodes subtending the lower foliage leaves which are undergoing most increase in girth and so build up the bulk of the corm. As a result the brown fibrous scales on the outside of the mature corm inserted near the base are scale leaves, but those inserted around the thickest part of the corm and around the buds at the top must be formed from the bases of the foliage leaves.

On the young corms the buds around the base of the dying flower stalk, that is in the axils of the upper leaves, will be enlarging and in these again small axillary buds will be just forming. Thus at this season four corm generations are actually present. It will be seen that if more than one bud grows out and stores food in its basal internodes, the crocus plant will be vegetatively propagated in this way. Besides the large buds at the top of the corm, some of the smaller ones lower on the flanks may grow, but usually these do not flower for the first few years and the same terminal bud will continue the growth in several successive years until it is finally used up in the production of a flower.

In the cases so far considered the organ of perennation has been a stem structure, and storage in this region of internodes, associated either with scale leaves or foliage leaves, may be connected with the fact that the internodes remain capable of growth for a long time. Associated with the other monocotyledonous character of long-continued basal growth of the leaf, there are numerous bulbous forms, in which the stem remains short and the food is stored in swollen foliar structures, the bulb scales. The way in which the monocotyledonous growth habit lends itself to the production of a bulb is well illustrated by comparison of the two allied plants, the leek

(*Allium Porrum* L.) and the onion (*Allium Cepa* L.). If a leek is cut in half longitudinally it is found to consist of a very short stem, on which are crowded a number of leaves, each of which is very long with a white cylindrical sheath surmounted by a long green blade, a ligule marking the junction of the two. The region of leaf sheaths is the edible part, as these remain growing and tender for a long time and are slightly swollen with stored food. In the onion the structure is similar, but a more definite bulb is formed, as the leaf sheaths grow less in length and more in thickness. When the green blades die down the basal parts in which the food is stored persist, and serve as a supply for the buds which grow out the following spring.

A more convenient type in which to start a more detailed study of the bulb habit is the daffodil (*Narcissus Pseudo-Narcissus* L.). The shoot of this plant produces each year on a very short stem the usual cycle of foliar structures, consisting of about three scale leaves, each with a basal sheath encircling the axis but the lamina scarcely developed, about three foliage leaves with entire sheaths surmounted by a long narrow lamina, and a foliage leaf with an incompletely sheathing base which encloses the flower stalk. Axillary buds are present in the axils of the two uppermost leaves. The complete cycle is represented diagrammatically in Fig. 25. All these structures contribute to the fleshy part of the bulb, including the base of the flower stalk. In the foliage leaves the blade dies down as far as a clearly defined level, which separates it from the swollen and persistent part of the sheath and from which it eventually breaks away leaving a scar. In the same way the flower stalk dies back to a clearly defined level and the distal part of the scale leaf also dies back, but in this latter case it is not shed, so that the swollen structure produced from a scale leaf may be recognised for a short time by the presence of the perfect apex ; it is also true that whereas the scale leaf sheath is of almost uniform thickness all round, the sheath of the foliage leaf remains thickest on the side below the lamina attachment. When a shoot grows up in the spring, it uses for its growth the food stored in bulb scales formed from the foliage of the previous season, but usually these are not entirely depleted, and the inner ones especially remain fleshy for a second season and thus add to the complexity of the bulb, whilst those outside,

from which all the food has been withdrawn, are recognised as thin, brown, papery layers. As the foliage of the current

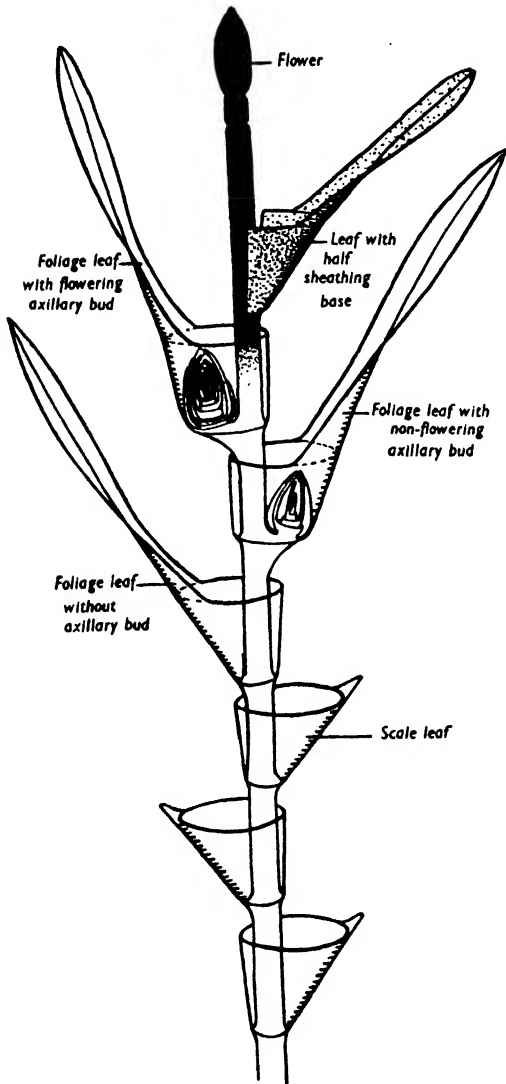


FIG. 25.—Diagram showing the typical annual cycle of structures present in the daffodil bulb.

season dies down, food manufactured by the green laminae is passed down and stored in the bases of the foliage leaves, the base of the flower stalk (if present), and also in the scale leaves of the main bud (if the bulb is not flowering) and of the axillary buds.

Of the two axillary buds of one growth cycle, that in the axil of the highest complete foliage leaf is a larger bud and normally contains the full complement of structures as already described, as shown diagrammatically in Fig. 25; it will produce a flower the following season. The bud subtended by the next lower leaf is rather smaller, and does not flower the next year. Both these axillary buds give rise to bulbs, so that very naturally

the daffodil bulb is a double structure, the two daughter bulbs being held together for a considerable time by the enwrapping bulb scales of former seasons. With this intro-

duction a daffodil bulb may be dissected, and the structures recognised and assigned to successive cycles of growth.

The recognition of the various structures in the bulb is facilitated if the bulb is dissected when the growth of the season

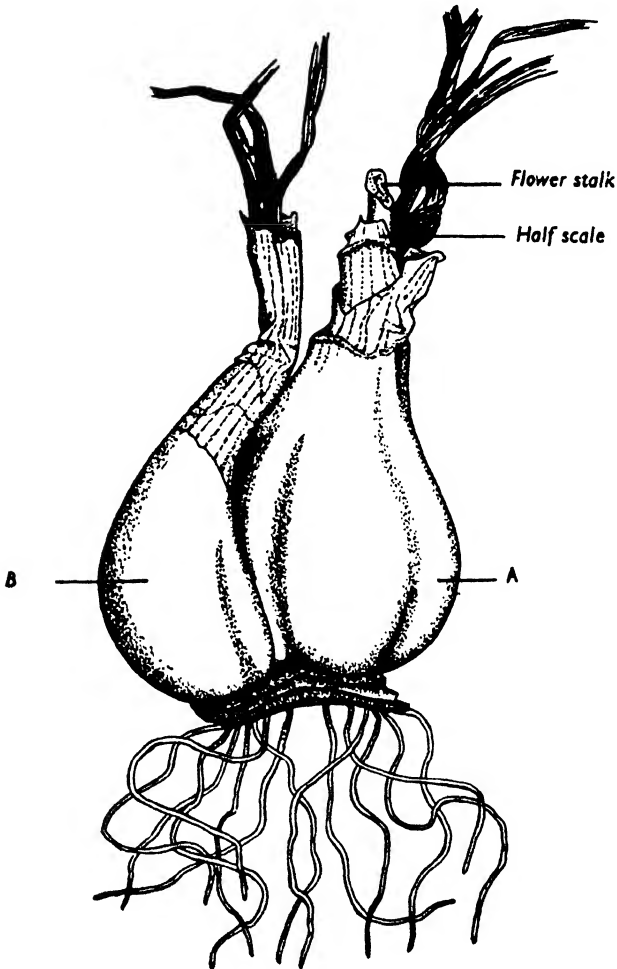


FIG. 26.—Daffodil bulb with papery scales and two fleshy bulb scales removed, showing daughter bulbs A and B still attached to the common stem ($\times 1$).

is completed and the foliage has just died down. The following description applies to a bulb dissected in July. The outer covering of the bulb consists of depleted brown papery scales which are so brittle that it is not possible to recognise the nature

of the parts with certainty. When these are removed the double nature of the bulb is seen as in Fig. 26, with a smaller bulb B closely adpressed to a larger A. The arrangement of the parts of this double bud are shown in plan in Fig. 27. Removal of B and of one scale from A reveals an incomplete bulb scale enclosing an old flower stalk (F), which was lying

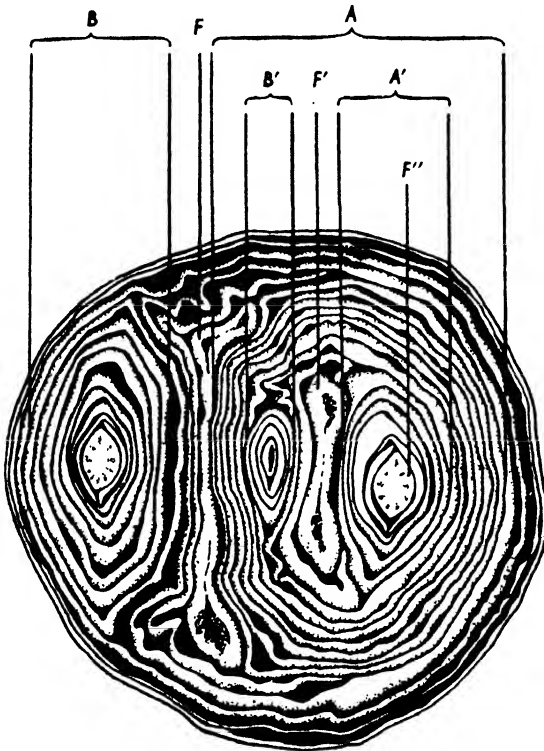


FIG. 27.—Daffodil bulb cut across after removal of papery scales ($\times 1\frac{1}{2}$).

between A and B as shown in Fig. 28*b*. The larger bulb A of Fig. 26 is shown in further stages of dissection in Fig. 28. The plan (Fig. 27) shows clearly that we are dealing with a compound structure, which is built up repeatedly in the manner already described. This figure represents a bulb examined in July, 1936, so that the structures present may be interpreted as follows :—

HERBACEOUS POLYCARPIC PERENNIALS 105

(At least 4 papery scales were removed before the bulb was cut across.)

- 2 encircling, partly depleted leaf bases 1935
- 1 " less " " " 1935 subtending bud B
- 1 " " " " " 1935 " " A
- 1 half encircling leaf base and included flower stalk . 1935

Bud B.

- Scales and foliage leaves . . 1936
- Scales, foliage leaves, and flower . 1937

Bud A.

- Scales and foliage leaves . . 1936
- Half scale and flower stalk . . 1936
- Axillary buds A', B' . . . 1936

Bud B'.

- 1 scale and 3 to 4 foliage leaves . 1937

Bud A'.

- 3 scales and 2 foliage leaves . . 1937
- Half-scale and flower . . . 1937

Fig. 28 shows stages in dissection of bud A. When the 1936 scales and outer foliage leaves have been removed, bud B' is seen enclosed in a scale leaf (Fig. 28c). Immediately within the next foliage leaf base lies the incomplete scale and flower stalk (F') of 1936 (Figs. 28d, e). Dissection of bud A' shows in the centre the flower bud (F'') for 1937 already present (Fig. 28f).

A bulb of a slightly different type is that of the tulip (*Tulipa* spp.). When tulip bulbs that have been growing in the soil are lifted, it is usually found that a number of bulbs are loosely grouped together, and have evidently arisen by vegetative multiplication from one original bulb. If a large bulb is selected from such a group it is found to be enclosed in a rather brittle, brown coat which is readily removed to disclose the swollen inner portion of the bulb (Fig. 29). Each bulb scale completely surrounds the bulb and tapers off distally to a short apex, to one side of which there is a relatively small opening through which the tips of the inner bulb scales may be seen. If these scales are split open and removed, it is found that there are usually about five or six similar structures, each one almost completely enclosing those within. They are all thick and opaque owing to reserve food stored in them, and the fact that a definite apex can be recognised on each scale makes it clear that in this case each swollen bulb scale is equivalent

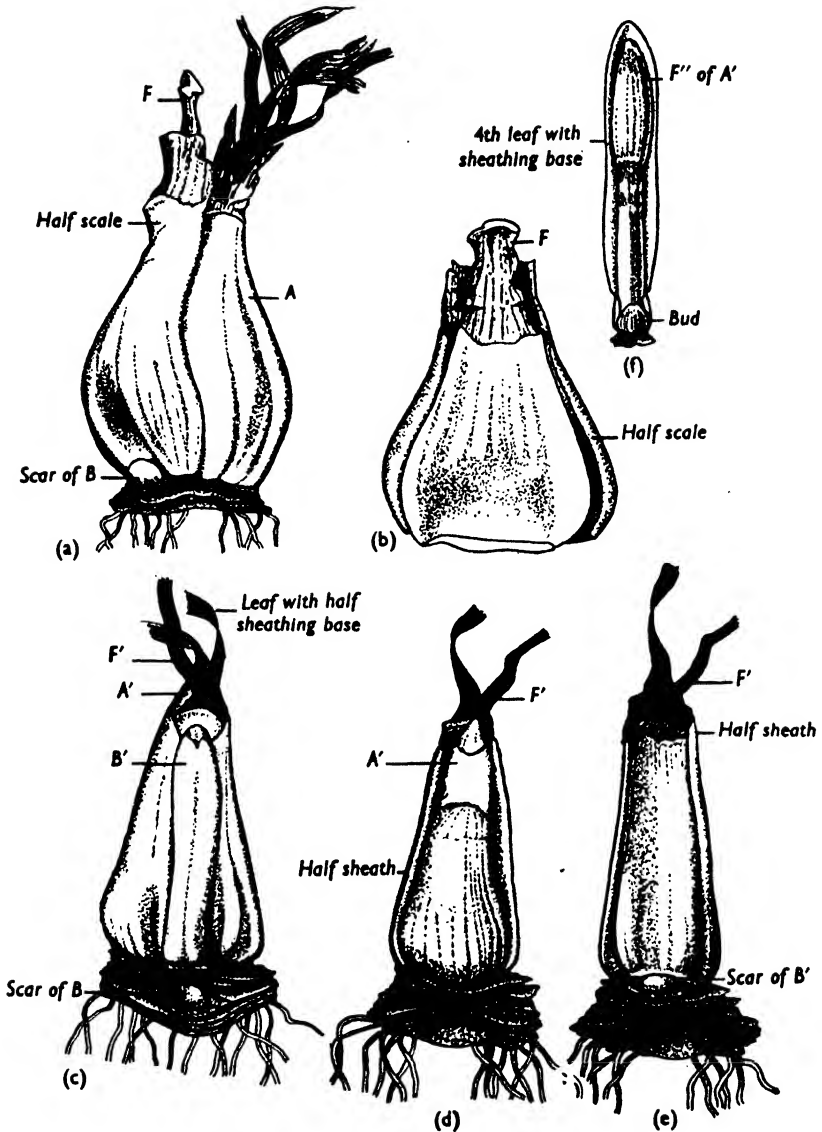


FIG. 28.—(a) Bulb of Fig. 26 with bulb B and one leaf base scale removed to expose the half scale enclosing the old flower stalk F and bulb A, (b) half scale and old flower stalk F, (c) bulb A with four scales and two foliage leaves removed, exposing bud B', (d) bulb A with one more foliage leaf base removed and viewed from the opposite side to (c), showing bulb A' with the old flower stalk F' and the half sheath behind, (e) the same as Fig. 28, (d) with bulb A' removed to expose the old flower stalk F' and half sheath behind, (f) bulb A' with scales and foliage leaves removed, except half leaf and flower bud F'' (a-e $\times 1$, f $\times 1\frac{1}{2}$).

to an entire scale leaf. When the fleshy scales have been removed the axis of the bulb is seen to narrow to a short stem region, from which may be dissected three to six leafy structures of a different kind. These are much smaller than the scales, yellowish in colour and longer relative to their width and are obviously the young foliage leaves, which in tulip are borne distributed along the erect stem which bears the flower. Generally a bud occurs in the axil of each swollen bulb scale, the most developed one is that which lies in the axil of the

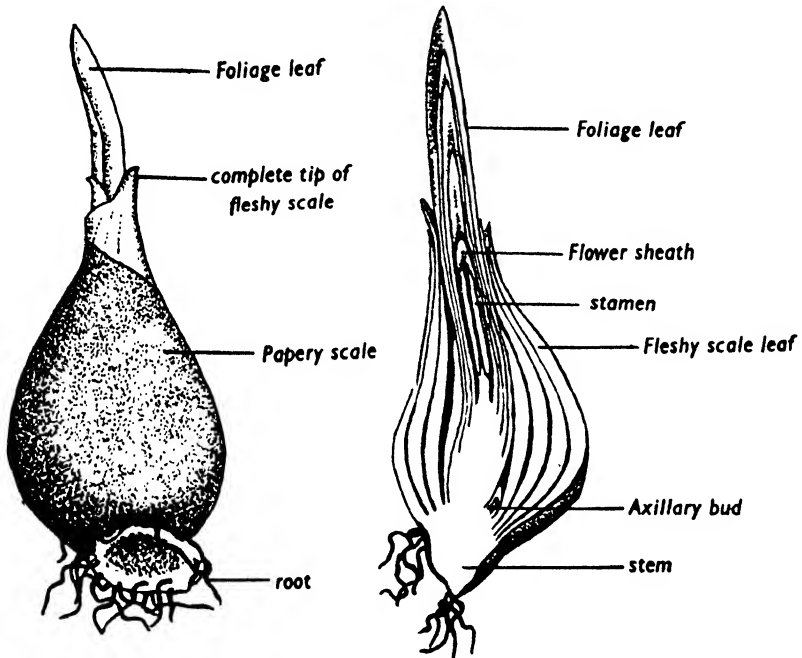


FIG. 29.—Tulip bulb entire and in median longitudinal section ($\times 1$).

innermost scale and which consequently comes to lie close beside the stem which carries up the foliage leaves and the flower terminating the axis of the main bulb. The bulbs are planted in this condition, and during April and May the foliage leaves and flower undergo a period of rapid growth and expand above ground. This process draws upon the food stored in the bulb scales, which gradually shrink and are quite depleted by the middle of June. Meanwhile the new crop of green foliage leaves is making food which is stored in the young scale leaves of the axillary buds, which consequently swell up into bulbs

during this period. If the bulb has had a central flower bud, the expansion of this uses up the main apex of the bulb, but in the subsequent season this is replaced by the bud in the axil of the innermost bulb scale.

When the axillary buds are recognised in the axils of the fleshy scales in autumn, they appear as small white structures with their outer scales swollen into small bulb scales. After the subtending bulb scales have been depleted during the next season's growth, the axillary buds are also found to have changed their appearance and to have become invested in a brown scale like the one enclosing the original bulb. This is actually their second season of development, since they were present the previous autumn, and the nature of the brown enclosing scale depends upon the way in which such axillary buds have behaved during the spring period. In some cases the bud which was recognised in the autumn simply continues to develop during the following spring, without expanding any foliage leaves, and as it undergoes this further development, its outermost scale is depleted of food and remains as a brown coat covering the young bulb. This is the more common behaviour of the buds and generally applies to the innermost bud, which eventually appears to carry on the original bulb. Occasionally, however, and more often in the case of the buds in the axils of the outer bulb scales, one of the foliar structures of the bud may develop a lamina and appear above ground; when this occurs it is the basal regions of such leaves which, when depleted, form the brown investment round the young bulb. Unless the brittle outer scales show an undamaged apex, it is therefore difficult to say whether it is equivalent to an entire depleted scale leaf or the base of a foliage leaf.

In bluebell (*Scilla nonscripta* Hoffm. et Link) (Fig. 30a), the bulbs dug up in autumn are white and fleshy, as the old depleted bulb scales rot away and disappear completely. As in former types the bulb scales encircle the bulb and in this case observation leaves little doubt that the outer swollen parts are the bases of foliage leaves, as the scar from which the lamina of the foliage leaf has died away is clearly visible to one side of the apex of each outer scale. Several swollen leaf base scales of this kind are present, but these cannot be dissected off cleanly from one another as they are fused

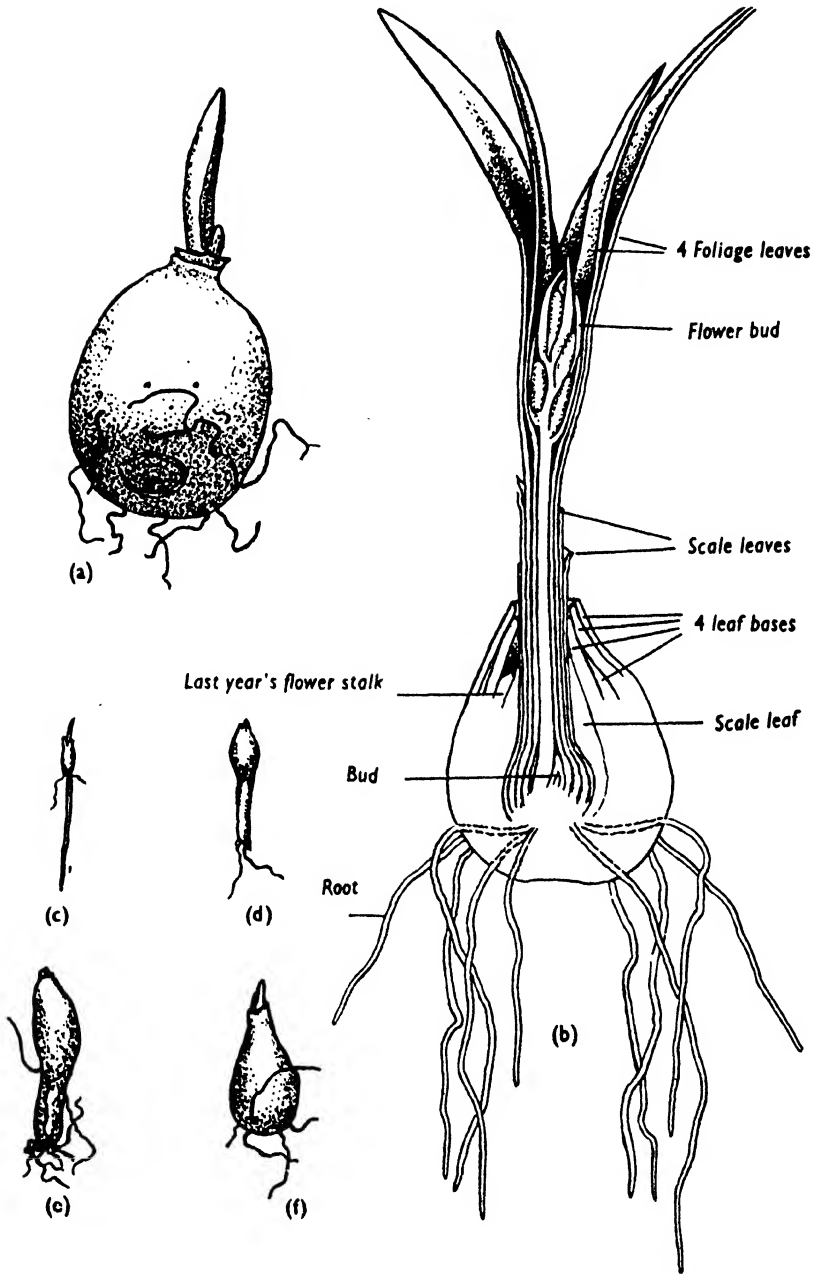


FIG. 30.—Bluebell, (a) entire bulb in autumn, (b) median section in March, ($\times 1\frac{1}{2}$), (c-f) stages in the development of bulbs from seed ($\times 1$).

together except for a short distance from the apex. It is also a point of interest to notice the way in which the new crop of roots arising from the stem are able to grow straight out through the outer fleshy bulb scales. When the fused leaf base scales have been removed from a well-grown 'bulb, a small narrow structure is exposed, which also has a distal scar; this is the base of the stem of the last inflorescence. It has close beside it the bud which developed in the axil of the uppermost foliage leaf and which will carry on the growth of the bulb in the following season. This bud contains the usual cycle of scale leaves, foliage leaves (with an axillary bud associated with the uppermost) and a terminal flower, and its scale leaves are already conspicuously swollen. Thus in this type the food is stored in the leaf bases and in the scale leaves of the next younger cycle. When the foliage and inflorescence are expanded in spring (Fig. 30b), the swollen leaf bases of the previous cycle and the scale leaves of the current cycle are depleted and disappear, and the outermost structures of the winter bulb are the leaf bases of that season.

In the first two types of bulb, daffodil and tulip, the uppermost axillary bud continued the growth of the main bulb, whilst other lateral buds developed into daughter bulbs and served as a means of vegetative multiplication; but in bluebell or hyacinth it is rare for more than one bud to develop, and the bulbs are therefore not multiplied to any appreciable extent in this way. If scales are removed from bulbs of *Hyacinthus* and put under conditions suitable for growth, buds may be produced from the bases of the scales, so that these bulbs may be vegetatively propagated by this method.

In bluebell, however, fertile seed is produced in the fruits and the seedlings grow into small bulbs. The seed falls on to the surface of the woodland soil and gives a seedling of the onion type, with an epigeous cotyledon and one foliage leaf in the first season; it is rooted by means of a stout radicle and a lateral root from the first node. At the end of the first season, the base of the cotyledon persists, though it only swells very slightly and the bulb consists mainly of the base of the first leaf swollen into a tiny bulb about 4 millimetres in diameter. During the next three years successively more leaves are formed, and as their bases and the young scale leaves within swell up, a bulb of larger size is formed each year and usually in the fifth year of

growth the apex of the main bulb is used up in the production of a flowering axis when the growth of the bulb in the following year has to be continued by the growth of a bud in the axil of the uppermost foliage leaf.

When adult bluebell bulbs are dug up from woods, they are found at an appreciable depth, often 5 or 6 inches below the surface, and they are often well embedded in hard layers of clay sub-soil. As the new bulbs are formed from seed shed on the surface of the ground, it is a point of some interest how the bulbs reach these deeper levels. Contractile roots play their part. The radicle of the seedling is a stout root which extends down well into the soil and subsequently contracts as it dies and becomes strongly wrinkled progressively from the base as it does so. The other roots formed from the first node are much thinner and are probably not important in this respect. For each of the first few years the seedling bulb forms one or more stout contractile roots of this type in addition to thinner roots. The contraction of the roots takes place as they begin to die in May, and by the second half of July the wrinkling due to contraction shows along almost the whole length of the thick roots, after which they die and are shed. This is also the period during which the leaf bases are swelling with the food passed down into them, and it is common to find amongst the small bulbs in the first years of their development a number of elongated bulbs, where the late growth of the leaf bases has been expended more on growth in length than thickness (Fig. 30c-f).

Some tulips also produce fertile seed, and again it is several years before the seedling has grown into the full-sized flowering bulb. Curious types of bulb are frequently formed, which at first sight are often puzzling to interpret. A single foliage leaf is formed for each of the first few years, and when the food passes down the base of the leaf may undergo extensive elongation downwards. Instead of the whole base of the bulb being carried down as in the bluebell bulbs, the leaf base breaks out through the enclosing sheaths and grows out like a pocket on one side, taking with it the central bud of the bulb, the axis of which is fused with the "dropper" on the side adjacent to the old bulb. By this means the bud may be carried to a distance of several inches from its original position, which is marked by the remains of the crop of roots of the old bulb. The scale leaves

of the bud swell up into the new bulb scales, and the new bulb is simply enclosed in the old sheath of the foliage leaf. Sometimes axillary buds in either young or flowering bulbs may also be carried some distance from the parent bulb by dropper formation and curious distorted forms may appear from lack of correlation in the growth of the axis of these lateral buds with the elongation of the sheath forming the dropper. Dropper formation is simply a further illustration of the remarkable power of growth retained by the basal parts of monocotyledon foliage or scale leaves.

In lilies the leaves on the aerial stem are rather exceptional amongst monocotyledons as they have a narrow insertion, and consequently, in some species, several are inserted almost at the same level and form a whorl. This feature is also exhibited in the type of bulb produced, where, although the scale leaves swell up, they do not enclose one another as in tulip, daffodil, etc., and give a bulb of a very different appearance. In many species the axillary buds both at the base of the stem or in the aerial parts may develop into new bulbs, but only those below the soil level swell up into normal bulbs, whilst those on the aerial parts remain small and are distinguished as bulbils.

The two types of bulb are often distinguished as tunicated (tulip, etc.), where the bulb scales are broad so that each completely envelops the younger ones within, whilst the bulbs with relatively narrow scales as in *Lilium* are described as corticated.

The characteristic of bulbous plants is that food manufactured by green, adult parts of the leaf passes down and is stored mainly in the foliage leaf bases (*Narcissus*) or the young scales (*Tulipa*) or in both (*Scilla*). Such food storage involves growth as the parts thicken up and persist, so that it must be closely associated with the faculty of long continued growth of the base of the leaf, a feature which is characteristic of monocotyledons, and it is only in this group that plants of the bulb habit occur.

The perennating storage structure produced in the case of bulbs, corms, or rhizomes, is formed at or below the ground level and the monocotyledons are particularly rich in earth-loving or "geophilous" forms owing to their manner of growth. The period of active aerial development of such

plants is relatively short, the rest of the year being spent in slow development below ground. Such plants, therefore, can survive the English winter, or the long periods of drought prevalent in Mediterranean districts or in the central tableland of South Africa ; geophilous monocotyledons are consequently richly represented in the floras of such districts.

CHAPTER IX.

THE WOODY PERENNIAL. I.

IN Chapter VII a distinction was drawn between herbaceous and woody perennials, and it was pointed out that the greater resistance to frost of the woody type was associated with the continued increase in girth of the older stem regions. A young leafy shoot of a woody perennial is quite similar in character to that of a herbaceous plant and collapses in a similar way when its tissues have been frozen, but the continuance of radial growth in this shoot is associated with a very definite change in its structure. These changes may be seen very clearly if a shoot from an elder (*Sambucus nigra* L.) is examined in early autumn before the younger herbaceous part has been damaged by frost. In this younger region the internodes are green because the green outer tissues, or cortex, are only covered by a thin transparent skin, the epidermis. A thin layer of harder woody tissue is found within the green cortex on cutting across the stem, and within this is a soft white pith. Towards the older part of the shoot the colour of the surface is seen to change from grey to brown, the grey appearance seems to be due to reflection from a layer of air beneath the loose epidermis, because when the epidermis is removed the cortex is seen to be as green as before. In the brown region the green cortex is covered by a brown layer (of cork) which forms at the surface of the green cortex but beneath the epidermis. At this stage the surface may be covered with a fine network of cracks as a result of the expansion of the tissues within, for a continuous ring of woody tissue of quite appreciable thickness is now present within the cortex, and the increased girth of the stem is largely accounted for by the growth of this woody tissue, though the pith may also be wider. In more typical woody shoots the woody ring thus formed bulks even more

prominently, and as in subsequent years more and more wood is laid down the stem becomes predominantly woody. The woody tissues are more resistant to frost, so that plants which have this characteristic of growth naturally compose a prominent part of the vegetation of countries with a severe winter season. Another feature of the growth habit of many of these woody plants is the short duration of the period occupied by leaf expansion and shoot extension. In many English trees, such as beech, sycamore and horse chestnut for instance, the leaves are fully expanded and the shoot extension has ceased as early as the end of June. During the warm months of July, August, and September, girth growth may continue and the shoot become progressively more woody and its outer tissues firmer and more corky. Although growth is actually proceeding at the growing points, there is little or no extension growth, but the buds are being formed in which will be found, enclosed within the bud scales, all the leaves which will expand the next growing season. The formation of the leaves in the previous season explains the rapidity of leaf expansion and shoot extension which takes place in the spring. The woody shoot with the leaf buds left upon it after the fall of the leaves in autumn proves fully resistant to the winter frosts.

All trees do not behave in the manner just described, thus the privet (*Ligustrum vulgare* L.) and lilac (*Syringa vulgaris* L.) continue to form more leaves whilst those already present in the bud are expanding, and the same is true of lime (*Tilia vulgaris* Hayne) and elm (*Ulmus procera* Salisb. and *U. glabra* Huds.). In lime and elm, and sometimes in privet and lilac, the later formed parts of the shoot dry out and wither during the warmer months, and the shoot dies back to older and more woody internodes which are more resistant.

In all woody perennials however, some part of the year's shoot growth becomes sufficiently woody to persist through the winter, and from this vantage point growth recommences the following spring, when new lengths of shoot are developed from some of the buds, whilst the older parts of the shoot system grow in girth. Obviously such plants will have a more prominent shoot system than the herbaceous types and give us the forms known as bushes, shrubs, and trees, which are of frequent occurrence amongst the dicotyledons and gymnosperms. Monocotyledons of tree-like stature, such as the

palms, are also found in tropical regions and may be grown in a few favoured localities in England, but these plants do not show a similar manner of girth increase and are not so frost resistant, and consequently they have not been included in the present discussion of the typical woody perennial.

Woody dicotyledons have relatively broad leaves and they also give a harder timber than do the gymnosperms (or conifers) in which the leaves are usually needle-shaped. The two groups are sometimes distinguished as broad and needle-leaved, whilst the timber merchant or forester may distinguish the same groups as hardwoods and softwoods respectively. Usually the broad-leaved dicotyledon has a deciduous habit (sheds all its leaves in winter); evergreen forms such as the holly (*Ilex Aquifolium* L.) are relatively exceptional. The reverse is true of the needle-leaved conifers, where most species are evergreen and only a few are deciduous, such as the larch (*Larix decidua* Mill.).

A Year in the Life of a Woody Perennial.

As the shoot of the woody perennial is thus built up of a succession of seasonal increments, we shall obtain a clearer idea of its construction if we follow the growth process in detail throughout one season in one particular example.

In sycamore (*Acer Pseudoplatanus* L.) (Fig. 31), the woody twig ends in a green bud which shows externally five to seven pairs of bud scales. In April the bud begins to swell, and this is quickly followed by slight growth and separation of the bud scales. The opening of the bud is mainly due to the fact that the scales expand more on the upper or adaxial side than on the lower or abaxial, and consequently after being incurved and folded over one another they become reflexed. The axis of the bud lengthens very little between successive pairs of scales, and as the latter soon fall off after the bud begins to open, there is left in their place a series of small, crowded, paired scale scars collectively known as the girdle scar. Within the scales are the foliage leaves which, like the scales, are borne in pairs, but become separated by elongated internodes, and undergo considerable expansion. The number of expanded leaves varies with the size of the bud, from about six to seven pairs in a vigorous terminal to only two to three pairs in a

small lateral bud. The foliage leaves expand quickly to their full size and then gradually darken to a deeper green colour and become more opaque in appearance, but otherwise remain unaltered from about the end of June to September. During this period, the new terminal bud and the lateral axillary buds increase slightly in size and are fully developed by autumn. The foliage leaves then gradually turn yellow and fall from the stem, leaving in the position of their former attachment the

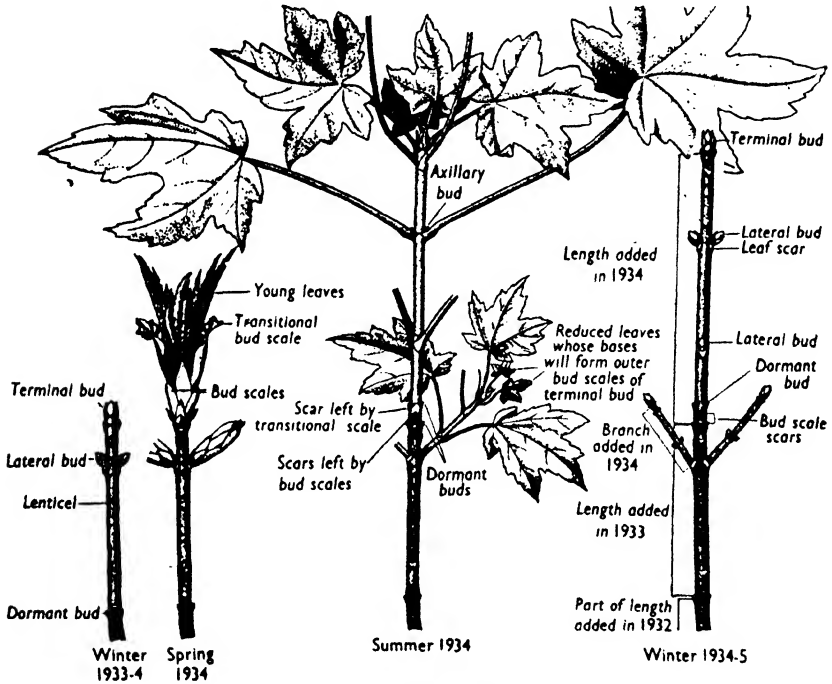


FIG. 31.—Sycamore twigs ($\times \frac{1}{3}$).

leaf scars which, from the first, are covered with a firm dry tissue. By this time the whole twig is brown, rigid and woody and bears only the buds. In this condition the twig remains through the winter. In the following season the terminal bud will go through the same series of changes and produce a region of crowded bud scale scars and an elongated foliage shoot, which is a continuation of the shoot of the previous season, whilst the lateral buds grow out into branches. After its first season of growth the woody twig undergoes no further

elongation, though it continues to grow in girth, and consequently the annual length increment is delimited on the twig as the distance between two regions of bud scale scars. These regions are easily recognised in such a type as sycamore, where the bud scales are numerous, but are not so readily identified in types with relatively few scales, such as willow (*Salix* spp.) or dogwood (*Cornus sanguinea* L.).

The new piece of extension shoot which develops each year from a bud resembles, in some respects, the shoot of a seedling. In sycamore it is usual to find that the internode which separates the bud scales from the first pair of leaves is shorter than the subsequent ones and similarly the leaves of the first pair are rather smaller than those of the next two or three pairs. The leaves of the last pair are also small as a general rule and may be of the type described as "transitional," when they are intermediate in form between typical foliage leaves and bud scales. This last pair of leaves is scarcely separated by any internodal extension from the scales of the next bud and also the scars left by their separation are deeper than scale scars but less deep than leaf scars. The greatest difference in the transitional type as compared with typical foliage leaves is seen in the reduction in the size of the lamina and in the length of the petiole. The outermost bud scales often show the remains of a minute lamina at the apex, and a comparison of the whole series of leaves and scales shows clear evidence of the fact that the scale is equivalent to the basal region only of the foliage leaf. As in the seedling, the axillary buds also show a gradation in size; those in the axils of the bud scales are small and normally remain dormant, whilst the largest and most vigorous are in the axils of the leaves of the largest pair.

In the particular case of sycamore, although the first year stem remains relatively green right to the base, there is a sharp line of demarcation between the stem surface, which turns dull and opaque as the cork forms beneath the epidermis, and the leaf base, which, with its petiole, remains light green and transparent. This sharp line is the future region of "abscission," where the leaf will be separated from the stem at leaf-fall and the clearer colour of the leaf parts is due to the absence of any cork formation at their surface.

On the stem surface numerous small marks are seen as rounded dots in the regions of the nodes and as elongated

slits along the internodes. These are evidently breaks in the epidermal layer and the torn edges are seen to be pushed up by the formation of loose powdery tissue beneath. Such breaks in the otherwise continuous layer of cork are known as lenticels. On the one year shoots of elder they are even more conspicuous than in sycamore, and here it is seen that they are already present on the internodes which are still green and herbaceous, as small brown dots. Below the first year shoot in sycamore the colour of the surface becomes more definitely brown, a change which, in the majority of woody plants, takes place in the first year shoot. The relatively green twigs and the green bud scales are among the distinguishing features of sycamore in winter.

Growth in Girth.

If the extension growth made by a sycamore twig in one season is examined it is clear that the girth falls off from the base upwards. The reason for this is seen very easily if the twig is cut across near the base and near the apex and the cut surface is trimmed with a sharp razor and then examined with a lens. As in the case of elder, it is seen that the woody ring of harder tissue round the pith is much thicker at the base of the twig, in fact it is so thin near the apex of the twig that it is not easy to distinguish it from the green cortex outside. If the shoot of the previous year is cut across in comparable regions and the surface is moistened with a solution of iodine in potassium iodide (this is usually helpful as any starch contained in the tissues is turned black by the iodine), the section from the base shows clearly that there is now a much thicker zone of wood and that this is composed of two rings, the inner of which stains darker in the iodine and which is comparable in thickness with the wood ring at the base of the first year twig, whilst outside this is another broad and less deeply stained ring. The section from the top shows a very thin inner wood ring like that from the top of the first year twig and this is surrounded by a new broad zone similar to the outer ring in the basal part of the twig. If such comparisons are extended to the older parts of the twigs, it would similarly be found that with each successive year a new zone of wood appears which must be formed to the outside of the wood of the previous year. When the extension growth of the new part of the twig is proceeding,

the basal internodes become adult and start to increase in girth first ; consequently at the end of the first season this process has been continued longer at the base and the zone of wood is thickest there. This effect, produced in the first year of its growth, is a permanent feature of the innermost ring of wood. The rings of wood added in subsequent years differ little in thickness along the length of this part of the stem in which no growth in length is taking place,.

The process of growth in girth is vigorous in the spring and is always associated with the commencement of growth in the buds. When the foliage is fully expanded, the formation of new wood slows down and gradually stops, not to be resumed again until the following spring. The annual ring, or ring of wood produced in the course of one growing season, shows a difference in texture if one compares the inner part of the ring, formed during the spring in its most vigorous period of activity, with the outer part formed later in the season, when growth was slowing down. The "spring" or "early" wood is less dense and lighter in colour than the "summer" or "late" wood. The region where spring wood formation starts actively after the winter, in contact with summer wood formed the previous season, is usually sharply marked by the change in colour and texture, so that the annual increment of wood is clearly defined. As such an increment is added to the wood each year, the number of annual rings seen if the twig is cut across indicates the number of years that the twig has been growing, and also agrees with the age of the twig at that level as judged by the number of rings of scale scars.

Since the laying down of a new zone of wood is an accompaniment to a burst of activity from the buds, it is only in such seasonal climates as the British, that the new increment should be described as an "annual" ring. In tropical countries, where the climatic conditions are suitable for growth throughout the year, or, in some cases, so dry that little growth may be possible for many years in succession, the rings may have no correspondence with years or they may be differentiated so little as to be scarcely recognisable. In a cacao tree (*Theobroma Cacao* L.) in Ceylon, twenty-two rings were present after only seven years of growth. The tree shed its leaves three times a year, and each burst of activity in the buds was registered by a zone of wood of the "early" wood type.

When wood is examined by inspection of the cross-section of a twig or of an older piece from the trunk of a tree, a difference may be seen between the wood of softwoods and hardwoods. In hardwoods, and especially in such a type as oak (*Quercus* spp.), the wood is characterised by elements in the wood which appear like pin-holes when cut across and which are particularly conspicuous in the early wood, whilst the late wood is denser and more uniform. If a softwood is examined in the same way, there are no such elements with large cavities, though the early wood is much softer than the late and cracks more on drying. The explanation of this difference is readily understood when the structure of the wood is studied with the microscope (see Chapter XXIV), when other differences between hardwoods and softwoods will also be discussed. In the hardwood, examined in cross-section, it will also be seen that there are radially running lines of a light colour, which cross the annual rings and are uninterrupted by them. These lines, known as rays, are also present in the softwoods, but are so fine as to be very difficult to see on the cut surface. Some of the rays run out continuously from pith to cortex, whilst others arise at varying distances from the centre of the wood and do not radiate so far. The continuity of the rays in a direct radial line through the wood suggests the fact, which will be confirmed later, that the wood is constructed upon a radial plan, and it is now seen further that as the rays may be followed beyond the wood in the same radial line, there must evidently be some other tissue, beyond the wood, which is also being built up in a similar manner. This is the phloem or bast, and observation of stems of different thickness will show that the bast is also increasing in thickness with time. The common radial plan on which wood and bast are built suggests that the same layer may be responsible for the growth of both and that it will be situated at the junction of both. The presence of such a growing layer, the cambium, may be confirmed with the microscope.

When radial growth commences in the spring at the surface of the wood, the bark (using the term in the general sense to include what may remain of the original cortex and the phloem, in fact everything outside the cambium) can always be peeled off the surface of the wood very easily, separation occurring in the region of the cambium, which, however, is only apparent

itself as a film of moisture on the separated surfaces. We have thus approached as closely as possible with the naked eye to a living, growing tissue, and as we shall also find at the shoot apex (Chapter XV), we find the tissue to have a soft, perhaps fluid consistency and to be almost as transparent as water. Evidently during the winter, when growth is not proceeding, either the cambium is not present or it is in a very different condition, for the bark is then firmly attached to the wood and there is no clean line of easy separation. This so-called "slipping" of the bark upon the wood in the spring is regarded by the forester as one of the best indications of the resumption of radial growth in the tree.

In addition to the radial growth of the wood and phloem, more growth also occurs nearer the surface of the tree. As the original skin or epidermis cracks, owing to the expansion of the tissues within, a cork-forming layer originates in the neighbourhood of the cracks and spreads from these points to form a complete layer. In some trees the same layer persists for many years and keeps pace with the increase in girth, and so in birch (*Betula pendula* Roth.) and plane (*Platanus orientalis* L.) the bark remains for a long time as a smooth, unbroken surface which flakes off later in wide, papery sheets. It is then evident that new layers have already formed within the outer sheets. In most trees, however, e.g. oak (*Quercus*), poplar (*Populus*), elm (*Ulmus*), etc., the first formed layers of cork crack early and continue to do so repeatedly, whilst all the time new layers of cork form beneath the cracks, so that an irregular, fissured bark is gradually built up. Evidently in such cases the bark must grow by the formation of new layers of cork, which arise deeper and deeper within the original cortex and then in the bast, and there would be no bast left in such trees in course of time if it were not that it is being continually renewed at its inner surface by the activity of the cambium.

A very striking feature of the radial growth of wood is its absolute dependence upon the growth of the buds above. If these buds do not grow for any reason, then the stem beneath the bud remains unthickened. This is the reason why, in the practice of pruning, the shoot is cut away just above a bud, for if instead a piece of internode were left above the last bud, no new tissues would form over the surface of the old wood in this distal internode. Tissues which are not growing

cannot be maintained healthy for long, so that eventually the internode withers and dies back as far as the next bud below. Such a "snag" of dead tissue is always liable to harbour disease, which, having gained a foothold here, may invade the healthy and growing tissue below. If on the other hand the cut is made just above a node with a healthy bud, when growth is initiated in the bud in the spring growth is resumed in all the tissues which lie beneath the bud, the wound remains healthy and a protective layer is formed beneath it in a manner similar to that healing the wound at leaf abscission.

From Year to Year.

This type of growth shown by the woody perennial in one year is, of course, repeated year after year. Each year, therefore, closes with a new crop of buds, raised farther from the soil upon a fresh set of branches, so that normally the woody branch system gets more extensive and more complicated every year. There are, however, certain factors at work, which bring about a change in the form and branching of the woody perennial in course of time. Let us consider, for instance, the fate of the buds along the length of a shoot developed in one season. The wych elm (*Ulmus glabra* Huds.) is a tree in which the shoots make considerable extension growth, so that the distal parts of the new shoots are still growing when a considerable canopy of foliage is already expanded. At this stage there is a great drain on the water supplies in the tree, and it seems that the necessary water is not available for the expansion of the distal regions. These immature parts of the twig are dried out and die back to a part which is more mature and woody. Just above a mature node a layer is formed across the twig which brings about the separation of the distal part, which is shed, leaving a small scar very similar to that left by the fall of a leaf. Thus in elm (*Ulmus procera* Salisb. and *U. glabra* Huds.) and in lime (*Tilia vulgaris* Hayne) and many other trees, the last bud on the twig will be seen to be flanked by two scars, one that of the subtending leaf and the other the scar left by the abscission of the last few internodes of the twig (Fig. 32a). A similar behaviour is observed in many of the terminal buds of lilac (*Syringa vulgaris* L.), syringa (*Philadelphus coronarius* L.), and privet (*Ligustrum vulgare* L.), where the terminal part of the shoot dies back and

is abscised in early summer. In these plants with opposite leaves the growth of the shoot is carried on the next season by the buds in the axils of the uppermost pair of leaf scars and

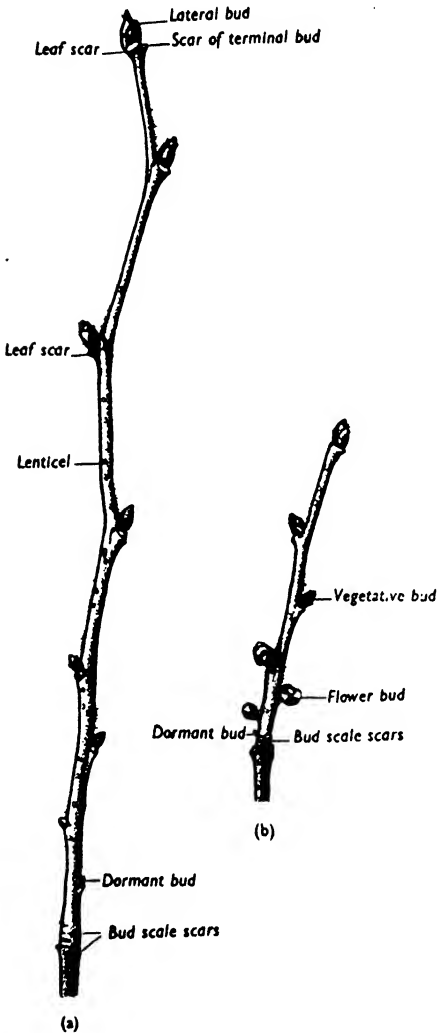


FIG. 32.—Wych elm ($\times \frac{1}{2}$), one year's growth, a vegetative twig, (b) flowering twig.

a forking of the shoot results. When growth is resumed the following season in wych elm, the extension of the shoot in approximately the original direction is now continued by the topmost axillary bud and the tree growth is sympodial. Many of the British hardwood trees show this type of sympodial growth, whilst in the beech (*Fagus sylvatica* L.), oak (*Quercus* spp.), and ash (*Fraxinus excelsior* L.), growth is monopodial, that is, the terminal bud resumes growth year after year. In horse chestnut (*Æsculus Hippocastanum* L.) and sycamore (*Acer Pseudo-platanus* L.) the growth is monopodial so long as the tree continues to grow vegetatively, but alters when flowering commences.

The wych elm shoot at the beginning of winter bears a series of buds along its length, all of which will probably be mature since the immature, distal regions have been shed early in the previous summer. Of these buds, the upper ones are of about the same size, but those formed in the axils of the lower leaves are smaller, and when growth

recommences in the spring, naturally the upper buds start to grow first. When these buds are growing vigorously they must make a great demand upon the woody branches below for water and food and, although there is probably a good supply, it is not unlimited. All the buds on the twig must be considered at this stage, not as partners but as competitors, and consequently those starting growth first are likely to make the most growth. Thus in elm and in most other woody perennials of the sympodial habit, the distal buds start growth first and grow into long shoots, whilst the lower buds develop into branches which are progressively shorter and stand at a wider angle from the stem as one passes down the shoot. Towards the base there are usually some small buds which normally fail to expand and are described as dormant. Such living but dormant buds may be of great importance to the tree in seasons of late frost, when all the growing and susceptible shoots may be killed. Under such conditions the dormant buds usually survive owing to their small water content and now, under suitable conditions and in the absence of stronger competing buds, they develop and clothe the tree with a later crop of foliage.

Consideration seems to suggest that, in spite of the size of the tree, the supplies available for growth of the shoots in the spring may be limiting. We have seen evidence that the gain in dry weight, which registers increase in food supply, is associated with the activity of adult green leaves. It is true that in each successive year, with the increasing branch system of the tree, the crop of such leaves is larger, but we see also that each year there is a larger woody branching system, all of which has to be clothed with a new layer of woody tissue ; each year also there must be a vigorous development of the root system below ground, or the complex shoot system above ground will fail for want of sufficient water supplies from the soil. When one considers that all this large growth system must be nourished by the activity of the foliage, it certainly would seem probable that in the course of years the supplies available for shoot growth in the spring may become more limited than before. As the tree grows older, we can see that the average length of the leafy shoots gradually diminishes and in some trees, as in the case of the poplars, there is a marked diminution in the size of the leaf. Indeed

both size and shape of leaf is often very different in seedling trees as compared with old trees.

There are many reasons for thinking that every shoot is very strongly individualised, so that for instance the food made by the leaves upon the different branches is mainly available for the further growth of their respective shoots. In beech (*Fagus sylvatica* L.) and some species of poplar the buds that are less well exposed to light make little extension growth but continue active for many years forming characteristic short shoots with crowded girdle scars. The shoot of wych elm may be taken to illustrate yet another point. The upper branch shoots are longer and more vigorous and have longer internodes, whilst some of the shorter branch shoots developed from smaller buds have very short internodes indeed, so that the leaves upon them are very crowded. In this case the leaves on the long shoots will supply food for the buds in their axils and also for the girth growth in the long internodes, whilst the crowded leaves on the lower shoots have most of their food available for their buds. Such an accumulation of food in a bud during its development is likely to lead to flower rather than leaf production and probably that is why we find in so many trees, as elm, birch, apple, etc., that the buds borne on these dwarf shoots, which have made very little extension growth, are the flower producing buds (Fig. 32*b*). The fact is of course of the utmost importance in the process of pruning a tree. When a grower wants to get the maximum amount of flower and fruit from an apple tree, for example, he wishes the major part of the food available for growth in the tree to be utilised for this purpose rather than for wood production. Therefore he cuts back the twig before growth commences in the spring in such a way that, after the first few buds have grown out into long shoots and the next few into short flowering shoots, there is no long region of bare wood at the base bearing only dormant buds. If such pruning is not carried out there will still be the production of long shoots at the apex and flowering shoots below, but beneath these there may be a comparatively long region of the branch, which bears only dormant buds, the whole of which has to be clothed with new wood at the expense of the food made by the leaves above.

It is an interesting fact that the distribution of leaf and

flower buds, described for elm, apple, etc., and which is very common amongst woody perennials, is hardly to be found in the herbaceous types. In the latter the shoot is usually found to be terminated by the flowering system and any leafy shoots still developing after flowering commences are found lower on the branches. If a glance is cast back to the discussion of the herbaceous perennial (Chapter VII), it will be seen how much this feature of continued vegetative growth of basal buds contributed to the perennation of the various types of plant found within the group. It is impossible to say, at present, how much significance should be attached to this difference in growth habit, but it may be recalled that a few pages earlier, the fact was emphasised that the renewal of radial growth in the woody shoot always began beneath developing buds. Such renewed radial growth is always more vigorous below a vegetative bud than a flower bud, so that if a vegetative bud terminates the shoot, then more wood will be formed in it at an early stage in development than if flower buds occur at the top of the shoot. Early wood formation in the shoot means earlier maturation of woody tissues and consequently a greater resistance to frosts, so that there may be some real significance to be attached to these characteristic growth habits of the two groups.

There are, however, a number of woody plants in which the terminal bud is the flowering bud. In particular, in trees with opposite leaves, the terminal bud lies between two well-developed leaves and it seems that the food resulting from the activity of these leaves is largely at the disposal of the terminal bud. The result is a large terminal flowering bud and two smaller lateral buds in the axils of the scars of the leaves of the last pair. Such a system may be illustrated from the horse chestnut, sycamore, lilac, guelder rose (*Viburnum opulus* L.), and many

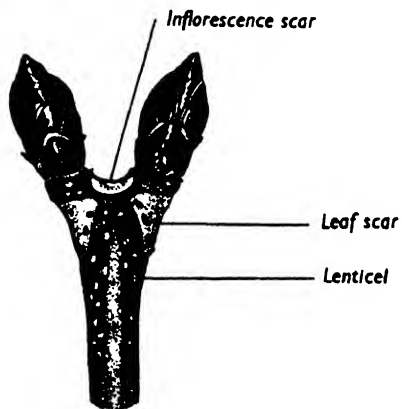


FIG. 33.—Twig of horse chestnut, showing the scar left by the terminal inflorescence ($\times \frac{1}{2}$).

other plants, and in every case the further development is the same. In the spring, the terminal bud exhausts itself in flower and fruit formation; the axis of the inflorescence makes very little radial growth and withers and falls, leaving a scar at the distal end of the shoot (Fig. 33). On either side of this flowering system, both the small axillary buds usually make some development, so that the shoot undergoes a dichotomous forking, but usually one of the resulting branches continues to grow more vigorously than the other, undergoes more thickening and becomes a main stem. Thus in these types the early vegetative growth is monopodial, but this is replaced by a sympodial habit as the tree begins to flower.

Flower buds, formed one year, usually grow out into flowers early the next year, and when they do so, they may draw so heavily upon the available food supplies that the growth of the vegetative shoots, which develop later in the same year, may be reduced in amount. This may lead to a biennial rhythm in the growth of some woody perennials; it is often shown well by trees such as the apple and pear, where in the year that the vegetative shoot development is limited by the heavy flower and fruit production, the leaf surface produced is not sufficient to supply enough food to cause the formation of many flower buds. The next year, as a consequence, most of the shoot growth is vegetative and vigorous and the activity of the large leaf surface encourages flower bud formation, so that the subsequent season is one of heavy flowering again. In this way the biennial rhythm continues, though some modification naturally results owing to variation in the seasons.

CHAPTER X.

THE WOODY PERENNIAL. II (*continued*).

Tree, Shrub, and Bush.

In the last chapter the apple was cited as exhibiting an internal rhythm between vegetative growth and reproduction, with the result that there is usually a good crop of fruit in alternate years. In some trees, however, the period of recovery from a heavy fruiting year may be longer. In the ash (*Fraxinus excelsior* L.), the "keys" or winged fruits, which are so conspicuous hanging from the branches after the leaves have fallen, are much more numerous in some years than others, and in the beech (*Fagus sylvatica* L.), the production of "mast" is vigorous only about once in five years. These differences are in part responses to weather conditions, but they are also largely the result of this internal balance between accumulation of food as the result of leaf activity and its exhaustion by flower production. There is, however, seldom any suggestion that the exhaustion of a tree as the result of heavy flowering may be so complete as to permit of no growth the following season and thus bring about the death of the tree. It is true that in old woody perennials the more distal buds may make only very little growth or even fail to start to grow at all, in which case the branches bearing these buds will die and the tree will become "stag-headed." But even in these cases some of the basal buds will almost certainly commence to grow, since being nearer the root system, they are in a better position with regard to the supplies of water and salts from the soil, and, freed from the competition of the distal buds, they often make very vigorous growth indeed and develop into straight, green shoots with long internodes and large leaves. Such basal shoots are known as "water" or "stool" shoots if they arise from the base of the trunk, or as "suckers" if

they arise from parts below ground. In some woody perennials it is characteristic for the growth of the upper distal shoots to diminish after a few years and for growth of the plant as a whole to be continued from more basal buds. Such plants form bushes, shrubs, or bushy trees, but never trees with a well-developed main axis or bole, which lifts erect a more branched distal "crown." Good examples of such woody perennials, which cannot be classified as typical trees, are elder (*Sambucus nigra* L.) and syringa (*Philadelphus coronarius* L.). In either of these it is striking to see how the branches fall off in the vigour of their extension growth after a few years and at the same time grow in a more horizontal direction. Such branches are replaced by erect growth from shoots arising from more basal buds. In these shrubs and bushes, where the active growth is continually being taken up by buds nearer the base of the plant, no appreciable length of well-marked trunk or bole is ever built up and only the part close to the ground thickens and develops a woody root-stock. In the tree the distal buds resume growth each season and the associated girth increase spreads down the same stem each year and gradually builds up the erect trunk.

Though the shrub or bush habit tends to give rise to a smaller plant than the tree, it is not necessarily shorter lived and in fact it is very difficult to put a time limit to the normal life of any woody perennial, whether tree or bush. A tree may be uprooted or destroyed by fire or frost, but unless completely killed by some such accidental means, death or injury of the main stem or main branches may be followed by the growth of dormant buds, which have been left buried in the bark near the base of the stem. In this way the individual plant may live for a very long period. Along the coast of California, the redwood trees (*Sequoia* spp.) grow to enormous heights, often well above 300 feet, and when the giant redwoods (the Wellingtonia, *S. gigantea* Torr.) are cut down, the number of annual rings show that they are often over 2000 years old, whilst a few specimens show over 3000 rings at the base. In the case of the redwood *S. sempervirens* Endl., when old trees die back and fall, a whole crop of vigorous suckers start into active growth from the roots around the old stump. At first these suckers compete vigorously with one another, but soon a few take the lead and a small ring of saplings grow up round

the old fallen tree. These saplings in their turn may grow into trees many hundreds of years old. Man has not yet been watching the process long enough to have a complete record of such long-continued growth activities, but the position of the trees as they grow in the natural redwood belt of forest often suggests that the trees are members of at least a second circle, thus surrounding an original stump now more than a thousand years dead, and there seems no reason why any limit should be put to this natural process of growth.

Amongst English trees perhaps the most outstanding examples are provided by the beech (*Fagus sylvatica* L.). In old trees growing in open situations, low horizontal branches may bend down until they rest on the ground, where they take root and at the same time give rise to a vigorous, erect shoot (Fig. 34). In this way the original tree becomes surrounded by a ring of rooted, vertical branches, which for all practical purposes are young trees, and these may continue growth should the original trunk fall.

Another remarkable tree of somewhat different habit, is the banyan (*Ficus benghalensis* L.). The seed of this curious woody perennial germinates on the bark of some other tree, where it has probably been deposited by birds. From the seedling the root grows downwards and branches on reaching the ground. The shoot system spreads horizontally and from time to time there grow downwards from it additional roots, which again branch on reaching the ground and ultimately thicken into strong supporting structures. In this way a single banyan tree may spread over a very large area, a single tree at Poona measured 500 metres in circumference.

Vegetative Propagation.

If then no limit can be put to the natural growth of the woody perennial, still less can one delimit the possibilities of its spread by artificial means. Some of the woody perennials root very readily from almost any portion of the stem. Thus most of the willows (*Salix* spp.) have already present in their woody stems minute root "initials," which may be seen with the aid of the microscope. If a piece of stem from such a willow is cut off the parent plant and put in soil or water, roots rapidly grow out from the basal end, whilst the upper buds develop into shoots, and thus a new bush or tree is derived

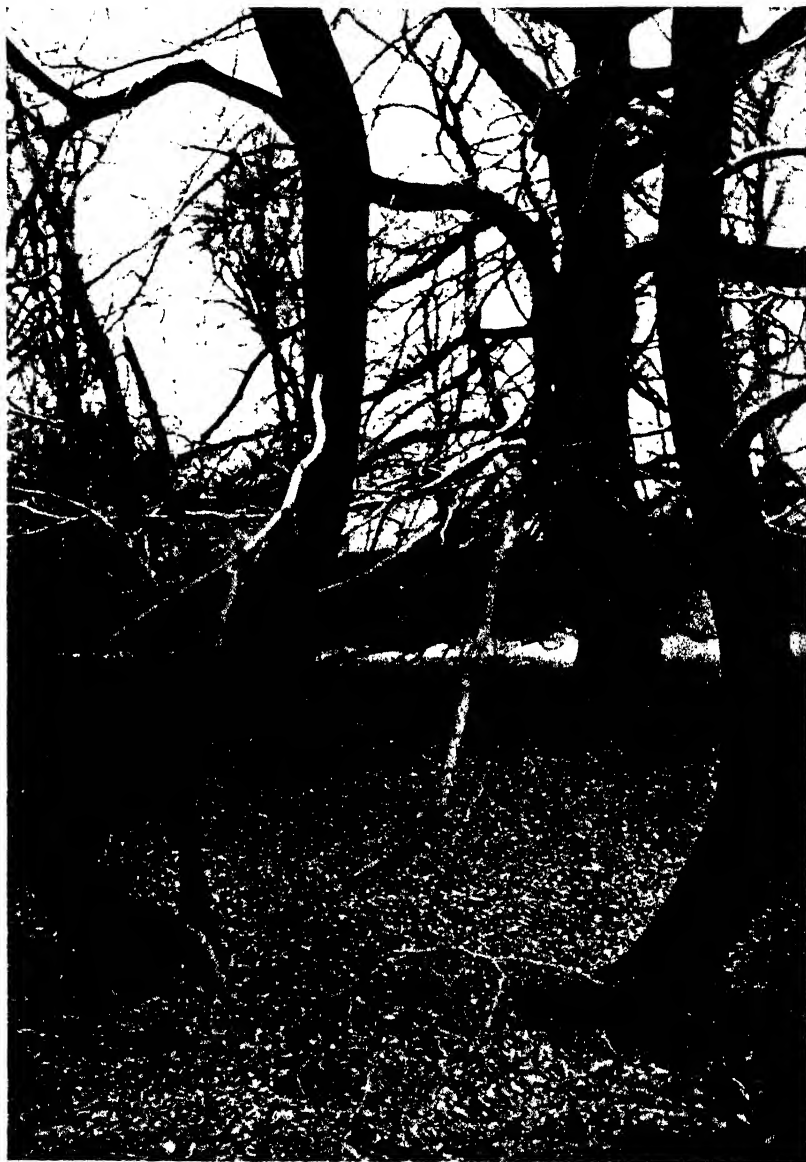


FIG. 34.—Layering beech at Potternewton, Leeds. In foreground, a slender branch strikes downwards from the trunk in the background ; the branch has rooted and on the left two thicker branches are seen to have arisen from it.

from the separated piece. In this way a whole bed of withies or osier may have been propagated from one individual of willow (*Salix caprea* L.) or basket willow (*Salix viminalis* L.). The gardener describes this process as striking a cutting and any woody perennial which roots freely from stems, e.g. the black currant (*Ribes nigrum* L.), may be propagated in this manner.

The root initials which grow out from the cutting may be already present when the branch is still attached to the tree, as in the willow. This is true also of some kinds of apples, where clusters of rootlets push a little way through the bark on the older branches and form excrescences known to the grower as "burr-knots." Burr-knots only occur on certain clones and many others, amongst which are most of those especially selected for the qualities of their fruits, never bear such rootlets on their stems and very rarely form them when cuttings are put into the ground. The result is that the grower, anxious to multiply a choice individual tree into a clone, has had to resort to the processes of grafting or budding. Branches or buds of the required variety are inserted as "scions" upon a "stock" of some other variety, of which rooted specimens can be multiplied freely. The latter are sometimes obtained by planting seeds, sometimes from cuttings of such clones as have the habit of rooting freely from severed branches.

In grafting, the operation is carried out in March or April, before the buds have started to swell. The stocks are often cut back even earlier in the previous January or February. The two main methods employed are "tongue" grafting, when the stock and scion are of about the same thickness, and "crown" grafting, when the diameter of the stock is greater than that of the scion. In tongue grafting, stock and scion are both trimmed with long, sloping cuts, in which corresponding notches are made so as to hold the two surfaces in position when they are placed together (Fig. 35). In crown grafting, the base of the scion is trimmed with a long sloping cut, whilst a vertical slit is made in the bark of the stock. The wedge of the scion is inserted between the wood and the bark of the stock and the two parts are bound firmly together until the tissues have grown to make a union.

Budding is carried out in July, when a bud on the new season's wood is cut out with a shield-shaped piece of the

surrounding bark attached. On the stock, a T-shaped incision is made through the bark, which is prised from the wood so as to allow the new bud to be inserted.

In either case the essence of the process for a successful union appears to be the fact that the scion and the stock both tend to grow in thickness. If a good union is made by the cambium which is laying down the new increment of radial growth, and provided that stock and scion prove to be "compatible," the junction of the two grows with remarkable uniformity and in the course of time it often becomes very difficult to see where the original graft was made.

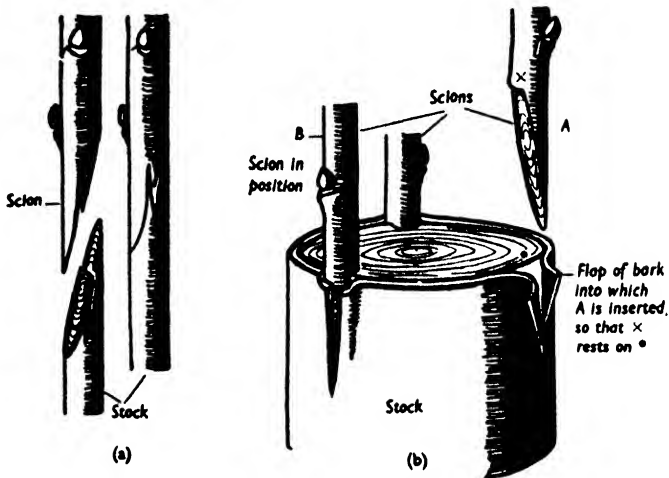


FIG. 35.—Diagrams of (a) tongue or whip grafting, (b) crown or bark grafting.

Since the establishment of the union between stock and scion is dependent upon the same growing region as is responsible for girth increase, it is natural that it should be possible to utilise the processes of budding or grafting for the propagation of plants belonging to the dicotyledons or gymnosperms, which undergo such girth increase. In the monocotyledons, however, where such growth in thickness of the shoot does not normally take place, it has not, so far, proved practicable to obtain a union by such methods. In view of the fact that the capacity for growth of a typical woody perennial appears to be unlimited, it is obvious that whenever a particular plant of this habit possesses properties of outstanding commercial value, it is worth while to explore very thoroughly

the possibility of multiplying this plant by vegetative means. In the Old World the methods of propagation by cuttings, layerings (when horizontal branches are pegged down, so as to induce rooting, and subsequently severed), budding, grafting, etc., now have several generations of experience behind them and practically all the large-scale fruit crops are propagated by some such means. In the New World, in the course of the last two or three generations, similar processes have been put into operation in the great orchard regions of the United States. At the present time the application of such methods is being investigated in relation to the important tropical crops, such as cocoa, coffee, tea, rubber, etc. This very brief discussion will have shown that the capacity of a piece of woody plant for independent growth varies with the plant and with the conditions under which the attempt is made, but the experience gained in temperate regions is full of promise for success in tropical regions, where skilled methods of propagation are only gradually replacing the primitive methods of the native cultivator.

Certain tropical crops, such as banana, banyan, and bread fruit, are normally propagated by vegetative methods. In the cultivated varieties of banana and bread fruit, it is extremely rare for seeds to be formed so that propagation is of necessity vegetative. The very occasional setting of seed in bananas is important however, as it offers an opportunity of obtaining new varieties.

The Tree Habit : Trunk and Crown.

Amongst timber trees, the dicotyledonous hardwoods and the cone-bearing trees or softwoods are readily distinguished by their branching habits, but they also differ in many other respects. The hardwoods bear their relatively broad leaves either singly or in pairs, and in the axil of practically every leaf a bud is formed, which is potentially able to grow out into a branch, though some of the basal buds on each year's extension shoot normally remain dormant. In the softwoods, on the other hand, the leaves are borne in a close spiral. In the axils of only comparatively few of these are branch buds formed and the more vigorous are grouped at the end of the year's shoot, so that when they grow out into branches, they appear to be arranged in a ring or whorl.

This grouping of the branches renders it easy to recognise each annual extension of the shoot system.

The characteristic of the tree habit is the possession of an erect trunk, which carries aloft a crown of branches bearing the foliage. It is evident that the form is influenced in large measure by external circumstances, since trees grown in open parkland have a comparatively short trunk region free from branches, whilst trees of the same species grown in the forest have a very long trunk region. Evidently in the forest shade the lower lateral branches on the trunk failed to continue growth. When buds emerge in the spring they are in competition with one another for the supplies of water and food in the tree, and the buds in the light, emerging first, may make such vigorous growth and draw upon these supplies to such effect that the buds in the shade on the lower part of the tree never commence growth at all. Twigs bearing buds that fail to grow will themselves not grow in girth; their supplies of water and food may then be drained away to other growing parts of the tree and such branches dry and die. In young forest trees the boles of the trunks may be thickly clothed with dead, dry branches, which are often cleaned off by the forester as the bases of all such branches are being gripped in the new wood growth of the trunk in which they will appear in future planks as knots.

Even if the forester does not remove the dead branches the tree may gradually clean itself. To understand how they disappear from the trunk region, it is simplest to think first of what will happen in the region of a branch union, when both main stem and branch are growing. Under the influence of the buds, as they start to grow, new radial growth will spread down the trunk and down the branch. In the hardwood, growth commences and continues to spread down both trunk and branches at about the same time, so that when the new growth down the branch reaches the branch insertion, the trunk tissues are usually just starting to grow at that level. In the region of the junction, it seems obvious that considerable adjustment of the tissues must be necessary, especially in the angle of the union, but the growing tissues are so plastic that the readjustment is usually made without any folding and a smooth and very uniform wood surface results. When planks are cut from a trunk of a hardwood so as to cut across branch bases, the

latter are seen as knots, in which the union of trunk and branch wood is very firm. In softwoods the new growth spreads down the trunk much more rapidly than it does down all but the uppermost branches. The result is that the wood of the bases of the lower branches is surrounded by a new layer of wood forming on the trunk before the new growth has spread down the branch to that point. This means that a good union of trunk and branch wood is only formed for a short time at the end of the growing season when trunk and branch are both growing simultaneously. Consequently thin softwood planks frequently contain knots which are loose and readily fall out. During the process of growth in girth the bark outside the growing wood is pushed outwards, but above and below the branch union it is also pushed on to a shorter vertical periphery. The hard outer layers of bark become sharply folded above the branch, and the same effect is seen in the development of a series of small transverse folds spread over a larger area below the branch.

After the lower branches have ceased to grow, the resumption of radial growth in the spring thrusts outwards the bark around the base of such branches, where it is still firmly held ; the latter then soon have the appearance of standing in narrow sockets. This is evidence of the violent strains under which the tissues are placed and an inspection of the lower branches, which are failing to make growth, will show that the folds in the bark around them are usually more accentuated than those around growing branches. Branches which fail to grow always lose water to growing regions and such dry brittle branches are, after a time, unable to resist the outward thrust of the growing tissues of the main axis. Ultimately they snap off and the continued growth of the main stem gradually buries the stub under new layers of wood. Over the surface of these new tissues a patch of new bark forms the branch scar. Around this scar persist the folds in the bark formed around the branch base, since the mode of growth of the bark retains the outlines of these folds, which are evident for many years in a smooth barked type of tree. Such a branch scar records the former existence of a branch at this point on the axis and signifies the presence of a branch stub, buried in the wood beneath and which would appear as a knot if planks were cut from this region of the tree. From the appearance of the branch scar something

of the history of the branch may be learnt. If the branch died when the tree was a sapling, the branch insertion would then occupy a considerable proportion of the circumference whilst the scar covering the stub would be small. Such branches will leave scars with the region of folding comparatively narrow in depth but very wide in proportion, as it still occupies the same relative proportion of the circumference as when originally formed. If the branch grew out comparatively late from a dormant bud, the scar will be small and the folds will occupy a much smaller proportion of the circumference. A branch that was broken off comparatively late in its life will leave a comparatively deep scar. Dormant buds are frequently seen amongst the folds around branch scars because the branch arises from a bud originally enveloped in scale leaves with buds in their axils. As the main stem and branch union grew in girth together, the expansion of the wood carried the bark outwards from the surface of the base of the branch, so that this region of the bark, bearing dormant buds, would come to lie over the surface of the wood on the main stem and would contribute to the folded region of the bark, when the dead branch was forced off. Some of these buds may make a small amount of growth each year and in association with this a little strand of wood is produced. If this strand of wood is followed inwards, it can be traced through the successive layers of wood, since formed on the main stem, until it reaches the stub of the branch buried beneath, with the wood of which it makes connection.

The patterns of the folds in the bark may be recorded from suitable trees by putting a piece of smooth paper over them and "rubbing" with cobbler's heel-ball, in the same way as inscriptions on old brasses are recorded by archæologists (Fig. 36).

The fate of the lower branches, which are cleaned off the bole of a forest tree as it grows in girth, has been followed in some detail because it illustrates so well the type of problem which is presented by the three-dimensional growth of a tree and the mutual adjustments of its tissues. Besides this general method by which the bole of a tree is cleared of laterals, certain kinds of trees shed large numbers of twigs by a different process. The ground beneath a well-grown black Italian poplar (*Populus serotina* Hartig.) in August or September is usually found to be

strewn with long twigs, which are often green and leafy at the apex and which have a characteristic convex base. The twigs are only occasionally branched, though the rings of bud scale scars show that they are often ten to eighteen years old, but in

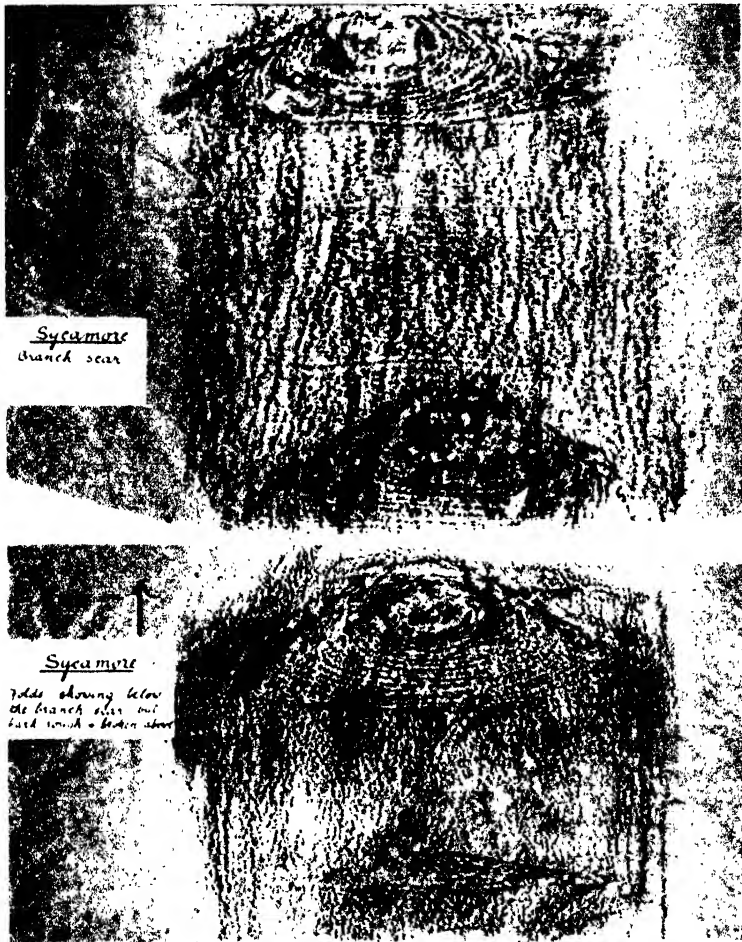


FIG. 36.—Photographs of “rubbings” of branch scars, white pointers indicate dormant buds ($\times \frac{1}{2}$).

every case the extension growth for the last few years has been very little. On the fallen twigs all the buds, with the exception of the terminal, contained only a group of male flowers surrounded by bud scales. In such a branch new radial growth

must depend upon the terminal leaf bud, because the catkins emerging from the other buds simply shed pollen and then fall off. The terminal leaf bud makes but little extension growth each year and the girth growth which it is able to initiate down the shoot is also very insignificant, so that no firm union is made between the wood of the branch and that of the main axis. The base of the branch swells out



FIG. 37.—Naturally abscised branches of oak.

in a characteristic way because a loose tissue is formed here instead of typical wood. Any strain, possibly even the weight of the branch itself, may cause the branch to separate from the tree in this region. As the tissues of the scar dry, the soft parts contract and radial cracks appear, which would not occur if a typical woody internode were cut across and allowed to dry. English oaks (*Quercus Robur* L. and *Q. petraea* Liebl.)

show a similar method of twig abscission, which is probably associated with flower production also (Fig. 37).

In poplars (*Populus* spp.) and willows (*Salix* spp.) weak vegetative branches fall off by a clean separation, which is somewhat similar to the abscission of reproductive branches in the previous types. In these trees vigorous shoots arise from the buds on the distal half of the previous year's extension growth, below this the buds develop into progressively weaker laterals until at the base only dormant buds are found. The weak laterals fall off in succession, the weaker ones going first. Thus on an older stem, we find the laterals grouped towards the distal end of each year's growth, the groups being separated by regions of bare stem furnished with branch scars or dormant buds. The age of a sapling or branch may often be gauged relatively easily by counting the groups of branches. The leafy crown is thus seen to be constructed on a systematic plan. The older "scaffold" branches in the centre are comparatively widely spaced, whilst lateral branches become progressively more numerous towards the surface of the crown, of which the periphery is composed of a crowded system of both strong and weak laterals, which together bear an almost continuous canopy of foliage. In other tree types in which no such natural abscission of weak laterals can be detected, a similar organisation of the leafy crown is attained by the gradual cessation of growth and death of the weaker, over-shaded laterals. These are then broken off and their stumps buried by the continued growth of the living branch system.

The few examples discussed do not exhaust the great variety of form shown by the woody perennial. They have, however, illustrated the fact that the form of the tree is very largely influenced by the close connection which exists between the extension growth from the buds and the radial growth of the branches beneath them. When we return to their study with the aid of a microscope, the general nature of these growth processes will be more fully analysed.

CHAPTER XI.

THE BUD SCALE. AN INTRODUCTION TO THE PROBLEM OF LEAF SHAPE.

IN the woody perennial new foliage emerges from the resting buds each year as the covering of bud scales falls. The range of form exhibited by the foliar structures expanded is very great, not only as one kind of plant is compared with another but even on an individual plant. It is no easy task to analyse the bewildering array of leaf forms, and it is hardly to be expected that any one general principle should be applicable to the interpretation of all cases. Up to now the approach to this problem of leaf form has been along two very distinct lines of thought, which may best be elucidated by examination of a specific case from both points of view. The bud scales are suitable for this purpose, for these structures, though clearly foliar in nature, have obviously become much modified in form as compared with the foliage of the same plant. This examination will show that whilst both points of view are suggestive, neither gives an adequate explanation of the multiplicity of form and structure with which we are confronted.

Most problems of form to-day are examined in the light of an argument as to the mechanism of evolution, that we owe mainly to Charles Darwin, and which may be briefly stated in the following form. Evolution means change, and Darwin has convinced most, if not all, contemporary biologists that species of organisms may change in the course of time. If amongst the variations so arising, some individuals are better fitted to survive than others, then these will be the ones to multiply, so that over long periods of time only those species will persist which have accumulated characters of value in enabling them to live under the prevailing conditions. Thus

in terms of this argument, the form of an organism is considered to be explained if it can be shown to be suited or "adapted" to perform a function. This is explanation in terms of function, a "teleological" explanation, which may be said to try and answer the question "Why?"

The second viewpoint concerns itself with the conditions under which a particular structure is developed and so attempts to answer the question "How?" such a structure came to have that particular form. As individuals of the same species of plant produce approximately the same series of leaf forms in successive generations, it is evident that the characters determining the general type of leaf are transmitted through the seed, but the variations between the individual leaves borne by the same plant may be determined mainly by factors operating during development. It is interesting to see if any light can be thrown on how these forces operate, especially since such observations may give some indication as to "how" a particular form and structure came into existence. It is obvious, however, that the difficulties in the way of analysis of the factors which affect growth during the successive stages of development are very great and our progress in interpreting leaf form along these lines will be slow. Such interpretations will not necessarily clash with the teleological view of the same structure but simply represent another way of regarding this problem of diversity of form; in the following pages the reader will have an opportunity to compare the two viewpoints and to form his own conclusions as to the extent to which they either arouse or satisfy his interest in such problems.

The Bud Scale.

The two viewpoints may be well contrasted in the course of a brief discussion of the nature of the bud scale. So long as a shoot is growing or is capable of growth, we expect to find at its apex a region where new leaves are still appearing and where the youngest are not yet separated by the development of internodes between them. Buds are only special cases of shoot growing points, and those occurring on woody plants in winter only differ in the fact that their growth is very slow during this period. In this resting condition the bud may also be characterised by the possession of "bud scales" which enwrap normal leaves within. The bud scales are distinguished

by the fact that they will not develop into typical leaves when growth is resumed in the spring, but fall off without undergoing much alteration in size or shape.

In the first place it is necessary to decide whether bud scales are legitimately regarded as leaves, in which case they are justifiably termed "modified leaves." From the teleological

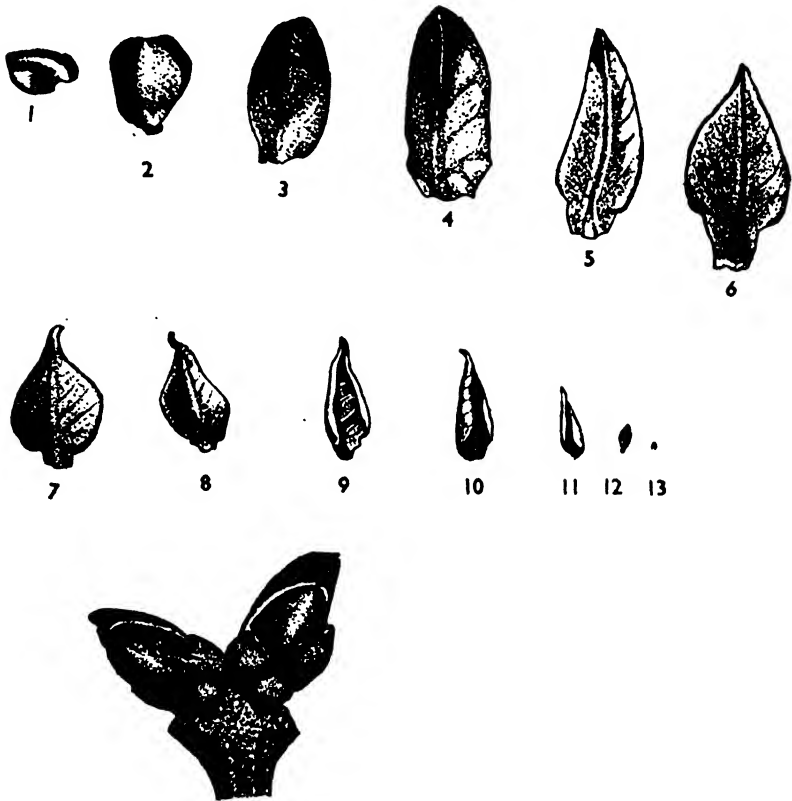


FIG. 38.—Closed bud of lilac and bud scales and leaves from a dissected bud, numbers 1-8 are drawn from the dorsal view ($\times 3$).

standpoint this raises the question as to the purposes for which they have been modified, and from the developmental standpoint how they came to be produced. Little doubt is left that the bud scales, like the first scale-like leaves on the bean epicotyl (Chapter IV), are to be regarded as leaves, if bud scales and leaves are compared during the process of development. The similarity in the arrangement of bud scales and leaves

and the presence of buds in the axils of at least some of the bud scales also allows of no doubt as to the general homology or fundamental similarity in nature of these structures. Close comparison, however, between the bud scales and the foliage

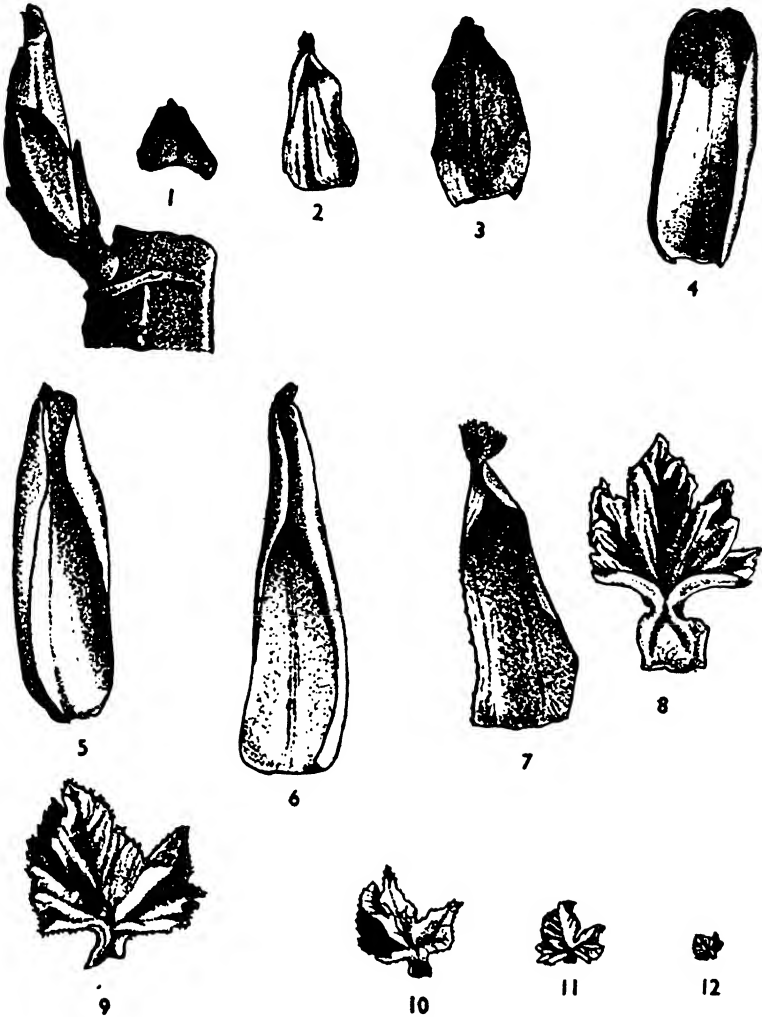


FIG. 39.—Bud of *Ribes* and bud scales and leaves from a dissected bud ($\times 3$).

leaves of the same plant shows that the two categories may bear various relations to one another in different species as regards the part of the leaf which appears to be represented by the bud scale.

The simplest case is that in which the outer leaves of the bud are only slightly modified. Thus in the way-faring tree (*Viburnum Lantana* L.), the buds are described as "naked"



FIG. 40.—Ash, photograph of bud scales, transitionals and leaves dissected from an opening bud ($\times \frac{1}{2}$).

as their outermost members are typical foliage leaves, which make appreciable growth in the following season. In such cases the annual increments of the shoot can only be recognised by the fact that the first internodes formed each year are very short.

In the bud of lilac (*Syringa vulgaris* L.), it is not easy to be sure how many bud scales are present as these are so much like the young foliage leaves, but as soon as the bud opens it is evident that the true scales are capable of very little further growth and also do not become separated by the extension of internodes between the pairs (Fig. 38). Such scales are evidently equivalent to entire foliage leaves and are described as "foliar." Foliar scales are also found surrounding the buds in holly (*Ilex Aquifolium* L.), rhododendron,

privet (*Ligustrum vulgare* L.), dogwood (*Cornus sanguinea* L.), etc. It will be noted that in all these plants, the foliage leaf has a relatively simple form.

A third type has already been mentioned in the case of

sycamore (*Acer Pseudoplatanus* L.), but is also illustrated by horse chestnut (*Æsculus Hippocastanum* L.), ash (*Fraxinus excelsior* L.), and flowering currant (*Ribes sanguineum* Pursh.) (Fig. 39). These are all types in which the leaf has a well-developed leaf base. Comparison of bud scales with the young foliage leaves shows clearly that only the leaf base is represented in the typical bud scales, whilst transitional forms often show stages in the reduction in size of the lamina and petiole (Fig. 40).

The fourth type occurs in plants where small lateral outgrowths, called stipules, are developed at either side of the leaf base. Examples of this type are apple (*Malus pumila* Mill.) (Fig. 41), laburnum (*Laburnum anagyroides* Medic.), mountain ash (*Sorbus Aucuparia* L.). The typical scales have a three-cleft

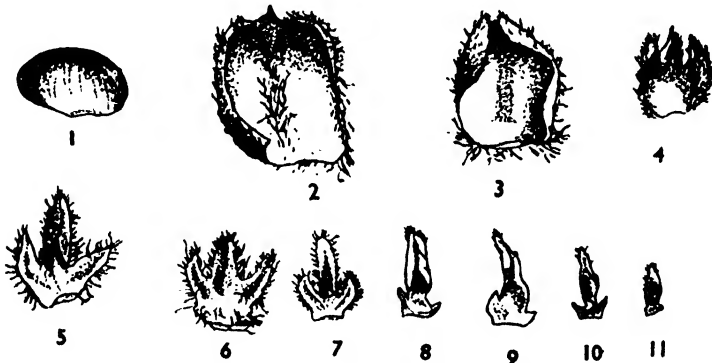


FIG. 41.—Apple, bud scales and leaves from a dissected bud ($\times 3$).

apex and the transitional forms show the gradual development of a lamina in place of the central tooth, thus leaving no doubt that the bud scales are equivalent to the pair of stipules fused to the leaf base. The stipules on the typical foliage leaves may wither and fall relatively soon after the expansion of the leaf, but they are visible on the young shoots in spring.

In some trees with stipulate leaves the leaf base is little developed and takes no part in the formation of the bud scales, whilst the stipules appear each as a distinct scale. In lime (*Tilia vulgaris* Hayne) the outermost scale is a broad, concave structure, which almost encloses the bud and is much larger than the second scale. Four or more pairs of scales may then be removed, but amongst these are seen the small, silky foliage leaves. With care it is possible to show that two scales are

associated with each foliage leaf and thus that each scale is equivalent to a single stipule. When this is realised, it is sometimes possible to break the bud transversely into a number



FIG. 42.—Lime, photograph of bud scales and leaves dissected from an opening bud ($\times \frac{1}{2}$).

of parts, each comprising a leaf and its two stipules. This fact is readily confirmed if lime buds are examined as they open in spring (Fig. 42), but if left too late the stipules are found to have been shed. Obviously in this type the bud scale scars will not form a conspicuous ring as in sycamore. In beech (*Fagus sylvatica* L.) the bud scales are similarly each equivalent to a stipule, but there are many pairs (about 9 or 10) before any foliage leaves are recognised amongst them and as no internodal elongation takes place between such pairs, they do ultimately leave a well-defined ring of crowded scale scars. Further within the bud, it is possible to associate two scales with each foliage leaf as in lime. Oak (*Quercus* spp.) has many stipular scales like beech.

From these examples it is evident that the bud scales are modified leaves, but with variation in the form of the leaf the part represented by the bud scale may differ. When only part of the leaf is developed as the scale, it appears always to be the basal part; the leaf base or stipules or both may form the scales whilst the lamina and petiole are suppressed.

Buds and Bud Scales.

There is in this country a tendency to associate buds enclosed in bud scales with winter, but it should be borne in mind that buds with bud scales are also formed in warm, wet, tropical countries, where conditions for growth are suitable all through the year. The impression has probably arisen from the fact that buds are more conspicuous when the leaves have fallen and in countries with sharply contrasted seasons, leaf-fall synchronises with the winter season. On the other hand in the tropics the leafy periods of the trees vary widely in duration ; some trees are leafless and others in full leaf, at all seasons, and it is not uncommon to find, even in the same tree, some branches leafless and some leafy at the same time.

However, the association of buds with winter has naturally led to the assumption that bud scales have a protective function, and that their presence enables the young foliage leaves inside the bud to survive the winter. Assuming the existence of such a protective function, the question arises as to the nature of the protection, when the first possibility that is suggested to one's mind is that the protection is against cold. This however will only be valid if there is some appreciable source of heat in the buds themselves, which a covering would help to conserve. Using a delicate thermo-couple the temperature inside the bud of an apple tree has been compared with that of the air around, when the results show that there is seldom more than 0.5° C. difference. During the day, if the sun shines, the bud rises in temperature more quickly than the surrounding air, but conversely during the night the bud cools more quickly and often registers a lower temperature. This is a clear indication that there is no internal source of heat in the bud and therefore no function for conserving such internal temperatures may be attributed to the scales. During the night, when protection would be most important, the internal bud temperature is actually lower than that of the surrounding air.

A further possibility is that the scales, which are often corky in nature and in many cases covered with resinous substances, might be effective in reducing evaporation. Though such a function is worth examination, in the English climate, where rain is so plentiful, protection against rain might be of almost greater benefit.

That the scales are not essential in all cases is shown by the resistance to winter conditions of the "naked" buds of the way-faring tree (*Viburnum Lantana* L.), and such buds as those of dogwood (*Cornus sanguinea* L.) and alder (*Alnus glutinosa* (L.) Gaertn.) where the scales often do not completely enclose the leaves within.

The protective function of bud scales can be tested to some extent by removing the scales from buds in autumn. Un-

TABLE 7.

Tree.	Buds descaled in November and collected the following June.					
	1930-31.		1931-32.		1932-33.	
	Alive.	Dead.	Alive.	Dead.	Alive.	Dead.
<i>Acer Pseudoplatanus</i> L.	—	11	—	17	—	4
<i>Æsculus Hippocastanum</i> L.	9	13	2	8		
<i>Betula</i> L. <i>pendula</i> Roth.					1	16
<i>Castanea sativa</i> Mill.	9	12	2	7	7	2
<i>Fagus sylvatica</i> L.	—	7			—	19
<i>Fraxinus excelsior</i> L.			2	1	9	2
<i>Populus canescens</i> Sm.	3	5	1	1		
<i>P. nigra</i> var. <i>italica</i> Moench.			1	3		
<i>P. serotina</i> Hartig					—	5
<i>Prunus Laurocerasus</i> L.					4	—
<i>Sorbus Aucuparia</i> L.			7	—	11	3
<i>Malus pumila</i> Mill.	—	4				
<i>Quercus Robur</i> L.	—	4	—	8		
<i>Rhododendron</i> spp.					—	4
<i>Ribes sanguineum</i> Pursh					8	4
<i>Syringa vulgaris</i> L.			5	5		
<i>Tilia vulgaris</i> Hayne	—	10	—	6	4	16
<i>Ulmus glabra</i> Huds.			—	5		

fortunately the operation of removing the scales, especially when they are very numerous, is a very severe one, and it is very probable that many of the buds which die after descaling may do so for this reason rather than because their protective scales are no longer there. The data in Table 7 gives some idea of the type of result obtained by this method, and it is interesting to see that in some species the buds seem little harmed by the removal of the scales.

Probably more important than the bud scales in determining

after shoot elongation and foliar expansion in May and early June little further growth is visible for some months. If the terminal and axillary buds are carefully dissected, it will

TABLE 8.

PERIODICITY IN LEAF DEVELOPMENT IN TREES.

Tree species.	Commencement of extension of shoot and expansion of foliage.	Behaviour of the growing apex.			
		Continued formation of leaves which will expand the same season.	Formation of scales.	Formation of leaves which will expand the next spring.	Commencement of rest period.
<i>Castanea sativa</i> Mill. Sweet Chestnut	May, 1934	May	Early June	July onwards	Oct.
<i>Fraxinus excelsior</i> L. Ash	Mid-May, 1934	None	Early and mid-May	Late May onwards	Aug.
<i>Ilex Aquifolium</i> L. Holly	Early June, 1934	Early June	Middle and end of June	July onwards	Oct.
<i>Ligustrum vulgare</i> L. Privet	End of May, 1934	Up to middle of June	End of June and early July	Mid-August onwards	Oct.
<i>Prunus Cerasus</i> L. Cherry	Middle and end of May, 1934	Until June	Transitionals early June. Scales mid-and end June. Transitionals early July	End of July onwards	Oct.
<i>Syringa vulgaris</i> L. Lilac	Mid-May, 1934	May	Early June	Mid-July onwards	Nov.
<i>Æsculus Hippocastanum</i> L. Horse Chestnut	Mid-April, 1928 (Foster)	None	Transitionals April 1-May 2. Scales May 2-July 4	July 4-Aug. 9	Aug. 9-April 1

be found that during the period of most vigorous leaf expansion scale leaves are forming at the growing points, which make very little progress during this time. During July and

August, when the leaves have ceased to grow, the buds enter upon a period of more vigorous growth and within the scales, which are already formed by this time, a new set of foliage leaves are formed, which will not be expanded until the following spring. We have already seen in considering the growth habit of the tree that one growing bud may be in competition with another, and that the vigorous growth of some buds may totally prevent the growth of others. Possibly in a similar way the vigorous growth and expansion of the leaves in the spring may so drain away the supplies from the growing point that the growth of the foliar structures forming there during this time may be relatively incomplete, and developing under these conditions they become the future scales. Later, when the leaves have ceased to expand and are probably beginning to return supplies to the growing point (as a result of the activity by which they add to the dry weight of the plant), then growth is once more vigorous at the apex. The structures formed under these more favourable conditions develop into leaf primordia which are able to expand into foliage leaves when they receive sufficient supplies of water in the following spring.

The data for some other trees are given in Table 8. It will be seen that in some types, foliage leaves may develop at the apex for a time in the early spring, before the drain of food supplies to the expanding foliage becomes too severe. Though this point of view seems very suggestive in considering the causes which link the internal rhythm of growth with the successive leaf forms that are borne upon a tree, the processes are as yet far too little understood and the data too scanty for any complete interpretation of the bud scale from the developmental standpoint.

CHAPTER XII.

THE INTERPRETATION OF LEAF SHAPE. THE GENERAL PROBLEM.

DISCUSSION of the special case of the bud scale has shown that interpretations of form need critical scrutiny, and this is particularly true of the wide diversity of form shown by the green foliage leaf. It is easy to suggest that the thin, flattened leaf of dorsiventral construction, which is the type most commonly found, is adapted to expose the maximum surface to light and air, in view of the fact that light and carbon dioxide are essential factors in the construction of organic matter by the plant. Nevertheless needle-shaped leaves and others of almost spherical shape are frequently found and evidently function successfully.

In fact, whilst the numerous species of flowering plants can usually be recognised by their foliage, leaf form being faithfully reproduced in successive generations, yet the wide diversity of form exhibited by plants in general, cannot be correlated with any obvious fitness of specific forms for various functions. In the following pages we shall consider leaf form almost entirely from the standpoint of development, but unfortunately this subject has been so little studied that the few ascertained facts cannot be used as the basis of generalised interpretations.

As an introduction to such developmental studies, it is helpful to dissect a large bud such as that of the brussels sprout (*Brassica oleracea* L. var. *gemmifera*). About fifty or sixty leaves may be removed from this bud, and these show a considerable range in form and consistency and supply a few relevant facts as to the changes occurring during development. The adult green leaves on the outside are attached to the stem by very short petioles which almost at once merge into the whitish midribs. On either side of this vein is attached a relatively

wide wing of lamina with a conspicuous network of smaller veins. Enclosed by the adult leaves are others which are yellower in colour and irregularly buckled. The vein system is still conspicuous but the areas of tissue in the meshes of the network are thrown into folds, convex towards the upper surface of the leaf and which are in fact only partially straightened out by the subsequent expansion of the leaf. In still younger leaves the buckling effect is not evident, though the vein system may still be seen. The leaves become pale in colour, very tender and watery in consistency and ultimately translucent, very soft and easily broken. Within these again, still smaller structures are present, which it is not possible to examine by simple dissection. It thus becomes evident that there is, in the centre of a bud, a tiny "growing point," on the flanks of which new leaves are continually being formed in succession. This fact is confirmed by experience, for leaves are never formed in any position other than at the growing apex of a bud. This suggests that the growth of the bud apex is mainly in surface so that a number of fold-like upgrowths succeed one another as the earliest stages of leaf formation.

In any plant the smallest leaf structures found nearest to the centre of a growing, vegetative bud are always extremely simple, and each consists of a bluntly pointed lobe, curved towards the apex or even slightly hooded, and which may give no suggestion of the form of the adult leaf. Such a structure is termed a leaf primordium. From the earliest time of its appearance this primordium is a mass of growing tissue centred round a vein. Veins are evidently channels of food supply so that it is perhaps natural to find that they are closely associated with the early stages of leaf formation, and very possibly the presence of veins in the axis of the bud is associated with the first emergence of the primordia in particular positions on the apex.

In the first place a general difference is often to be observed between the behaviour of primordia of monocotyledons and dicotyledons; in this connection, however, it is difficult to make any rigid statements as exceptions will be found to almost any distinction that one may draw. In both types the very first emergence of the primordium is similar, but in most cases the localised growth which leads to the erection of the fold-like

primordium is more restricted in the dicotyledons, whilst in monocotyledons the growth of the apex, followed by the upfold of the tissues to form the primordium spreads all round the axis and into this wider primordium numerous veins from the axis are continued. Also in the monocotyledon it is the further growth, as a unit, of the primordium thus laid down which gives rise to all parts of the future leaf. In the dicotyledons on the other hand, though the insertion may in some types extend all round the axis of the bud, more often it is restricted to one half or less of the periphery, and the veins entering this narrower primordium tend to be fewer, often one, three or five. From the primordium again develop all parts of the future leaf, but it is a striking feature, in contrast to the monocotyledons, that the growth is much more localised round the veins and the lamina is mainly developed by a later growth of wings of tissue which develop from the margins of the finger-like upgrowths, localised around the main veins.

The form of the young dicotyledon leaf, and especially of the lamina which is the first part to show conspicuous development, varies greatly amongst different plants, but a few examples may illustrate some of the many possible lines of development. In privet (*Ligustrum vulgare* L.) (Fig. 43a) the primordium continues to grow in length and thickness as a simple, slightly hooded lobe around a strand of vascular tissue. In sycamore (*Acer Pseudoplatanus* L.), (Fig. 43b) one vein enters the primordium and becomes the midrib of a central fingerlike upgrowth, this is shortly followed by two lateral veins, which diverge from the midrib almost at the base of the primordium, so that the localised upgrowths around these are distinct from the median, and the very young leaf is 3-lobed. After this two other lobes are formed in a similar manner.

In ash (*Fraxinus excelsior* L.) (Fig. 43c) the young primordium grows mainly in length, but from this there soon appear lateral lobes, which arise progressively from the base towards the apex. The vein supply into the primordium consists of an arc of separate veins, which enter the rather narrow insertion and run as a common midrib for a time, but subsequently diverge, causing the formation of the lateral lobes. These few cases illustrate the effect of the very localised growth around the veins in the dicotyledons, where the finger-like

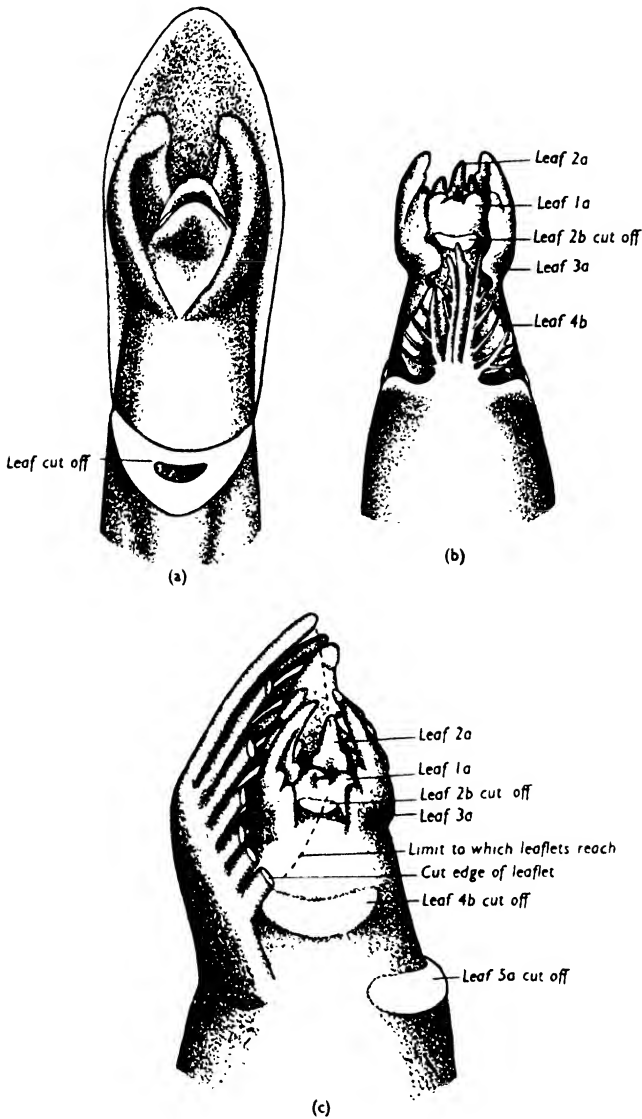


FIG. 43.—Diagrams of magnified buds. (a) Vegetative bud of privet as seen from the side and with the older leaves removed. (b) Vegetative bud of sycamore as seen from the side. (c) Vegetative bud of ash as seen from the side with leaves removed to expose the apex and youngest primordia. (The internodes are drawn with the length exaggerated to enable more detail of inner primordia to be represented.)

upgrowths increase in thickness and follow the pattern of the vein supply.

The main directions of vein development are determined very early, and a lamina in which all the main veins diverge from the base tends to give rise to a broader leaf than a type in which branch veins diverge in succession from the median vein. The early stage of localised growth around the veins is followed by the different type of growth, which takes the form either of a wing-like growth from each side of the veins or of a continuous sheet joining the veins, rather like the webbing joining the toes of an aquatic bird. These two phases of growth of a dicotyledon leaf are well illustrated in the development of the sycamore leaf. At first the developing lamina has somewhat the appearance of a microscopic hand with its five fingers (Fig. 43*b*), but when the "webbing" type of growth commences, this unites the diverging lobes into a continuous sheet, though the greater growth around the veins still continues so that five lobes project around the margin marking the positions of the main veins. Subsequently the growth between the veins becomes very vigorous and as this surface growth cannot be accommodated in one plane, it becomes thrown into sharp folds between the veins. As this tissue grows, there develops within it a system of branch veins, which forms a fine network throughout the whole sheet. The ultimate branches may end blindly in small patches of lamina often spoken of as "vein islets," when the type of venation is described as open, since free vein endings are present at the margins and throughout the tissues. In such a type the growth tends to be relatively simultaneous over the whole surface of the lamina.

Details of development in the leaves of dicotyledons are exceedingly varied, but the final form represents the resultant of the two phases of growth. If the wing development keeps pace with the growth around the vein system, a lamina of simple outline results, and in this the more marginal veins may be linked up with one another around the margin and to the median vein at the apex of the leaf to form a closed system. Frequently, however, the growth continues to be more vigorous around the veins and the particular outline will depend upon the plan of venation; if the main veins diverge from the same point near the base of the lamina the system is described as palmate, if lateral veins diverge in

succession from the mid vein, as pinnate. At the leaf margin localised growth around the tips of veins produces saw-like serrations, which are curved towards the leaf apex in the longer leaves or leaflets with pinnate venation, whilst wider leaves with palmate venation usually have a margin with more symmetrical dentations, a frequent forking and linking of the veins near the leaf margin may be associated with a crenate margin. Very rapid growth around the main veins often leads to a lobing of the lamina, or in other cases separate lamina expansions may develop around the main veins giving rise to a compound leaf with several leaflets. Thus in dicotyledons all gradations of leaf dissection are represented, most of which may be described as of the ternate (three main veins), palmate or pinnate types, the leaves being either simple, lobed or compound.

In monocotyledons a larger number of veins enter each primordium, of these the median is associated with the localised growth which causes the first emergence of the primordium, but usually the whole periphery of the shoot apex soon contributes to the formation of the primordium and more marginal veins enter the same primordium in succession. The growth in length of the primordium is very rapid, whilst the increase in thickness in the immediate neighbourhood of the veins is usually less marked than in the dicotyledons. The first few veins to enter the primordium are continuous to the apex, where they are linked together; additional longitudinal veins may subsequently appear, but these also are linked up around the margin of the lamina so that, with few exceptions, the main veins form a closed system, converging to the apex. Besides the main veins, intermediate longitudinal veins and a system of transverse connecting ones develop in the growing lamina tissue between the main veins, and this later vein development always proceeds in basipetal order.

The vein system thus built up in the monocotyledon lamina is a closed system, in which free vein endings only occur exceptionally, as in black bryony (*Tamus communis* L.). In young monocotyledon leaves there is little evidence of vigorous growth along the margins of the veins as in the dicotyledons, but the growth seems to be especially vigorous in tissue into which veins are just developing. Thus growth is at a standstill extremely early at the apex of the lamina, which consequently

remains narrow and pointed, but nearer the base a zone of vigorous growth is present in front of the smaller longitudinal and transverse veins, which develop as a system from the leaf apex in a basipetal direction. This long persistent basal zone of active growth, which is responsible for the great growth in length of many monocotyledon leaves, as well as a considerable degree of growth in width, is one of the most consistent features of monocotyledon leaves and one in which they differ from the dicotyledon types with more uniformly distributed growth over the whole lamina.

The presence of numerous longitudinal veins in the leaves of monocotyledons, which are often long and comparatively linear in form, has given rise to the term "parallel" veined to describe the general feature of the venation. As, however, a very general fact about the longitudinal veins is that they are linked at the apex of the lamina, the term seems far from appropriate and might be replaced by convergent.

In the leaves of monocotyledons some degree of variation in form is also shown. The leaf always tapers to the distal region of the lamina, which was formed first and became adult first, but behind this point the lamina widens towards the basal growing region and it is due to the activity of this region that the variation in form is mainly due. If the growing region soon attains its maximum width and grows only in length, a long narrow leaf is developed, but if the growing region becomes progressively wider, the longitudinal veins are carried apart and follow a curved course, as in water plantain (*Alisma Plantago-aquatica* L.). In more extreme cases, as in water arrowhead (*Sagittaria sagittifolia* L.), the basal growth is excessive and appears as two basal lobes to the lamina (Fig. 45). In the extreme case of the palms excessive surface growth takes place between the longitudinal veins, causing the leaf to be thrown into sharp folds. When such a leaf finally expands the lamina breaks along the folds, the marginal vein is torn off and the other veins, freed from their linkage at the apex, diverge so widely that the tissue of the lamina is torn apart between them. In the two leaf types of the fan and pinnate palms compound leaves thus result, but these are obviously of a different type from the compound leaves of dicotyledons. Throughout development in the convergent vein system of the monocotyledon there is present

a continuous unbroken vein running just within the margin from base to apex of the leaf. Under these circumstances naturally the margin of the monocotyledon leaf is usually entire.

Up to now little has been said about regions of the leaf, other than the lamina. All parts of the leaf arise from the primordium, but in the dicotyledons this becomes differentiated quite early into a basal part, which may form a conspicuous leaf base, and an upper part which forms the lamina. Most frequently, wing growth is confined to the more distal part, whilst a narrow region, which grows mainly in length and in thickness, may develop between leaf base and lamina as the leaf stalk or petiole. The petiole is usually late in extending so that the lamina is formed and folded in the bud close to the leaf base and does not expand until it is subsequently carried out by the extension of the petiole. Occasionally wing development may continue down the petiole and even down the internode, as for example in potato (*Solanum tuberosum* L.) or *Helenium*, but in such cases it is usually less developed than in the lamina. When stipules are formed, they usually appear very early as lateral lobes at the base of the primordium and are distinct from the more central lobe which gives rise to the lamina.

In many simple leaves the midrib is a direct continuation of the petiole and the distal part of the midrib alone is clothed with the wings of lamina. In certain other leaves, and perhaps especially in palmate types, the lamina is developed at an angle to the petiole and also behaves in development as an independent unit. This is true of sycamore (*Acer Pseudo-platanus* L.), and it will be observed in such types that the wings of the lamina show no tendency to extend down the petiole, but in some, as the lamina grows, it may join up into a continuous area of tissue, which completely surrounds the union of petiole and lamina. This is illustrated by the castor oil (*Ricinus communis* L.) or in the rounder peltate leaves of the garden nasturtium (*Tropæolum majus* L.). A somewhat similar effect may be seen in a few plants with leaves which have no distinct petiole. On the elongated stems of sorrel (*Rumex Acetosus* L.) the leaves have no petiole, and the young lamina grows so vigorously that the basal parts extend beyond the leaf insertion as "auricles" which clasp the stem. When a pair of such leaves are inserted at the same level, they may

fuse during development into a continuous collar round the stem, as in honeysuckle (*Lonicera Caprifolium* L.) or *Claytonia perfoliata* Donn., when they are described as connate.

As in the dicotyledon the whole leaf of the monocotyledon develops from the primordium, but the lamina is the first part to show conspicuous development. The basal part of the primordium remains very short and undeveloped whilst the lamina is growing, and it is only when the basipetal vein system has developed throughout the lamina that the basal part undergoes extension to form the leaf sheath, which often encircles the axis. In some cases the lamina is developed at a slight angle to the sheath and tends to spread out from the axis as it grows, and at the junction of sheath and lamina in many monocotyledon leaves, as for example all those of the grasses, a small upgrowth develops relatively late and continues the line of the sheath; this is known as the ligule. Sometimes a "stalklike" region connects the expanded lamina to the sheath, as in *Aspidistra*, but this region is very differently constructed as compared with the petiole of dicotyledons. Similarly in the monocotyledon lamina there is often a thicker median region, which simulates the midrib of dicotyledons, but this is usually a region in which the longitudinally running veins are more crowded and no one vein can be singled out as the large midrib vein which has caused excessive localised growth. When a group of closely running veins is present, this region may form a thickened extension below the expanded region of the lamina before the veins become more distributed in the thinner sheath, and it is the continuation of this "midrib" which is thus responsible for the "petiole."

It is obviously impossible to deal, in a short discussion, with the multifarious forms exhibited by leaves and still more to give any adequate explanation of them. The general type of leaf is obviously a specific character, since any species of plant will bear leaves which only vary within a certain range. For example the same shoot of passion flower (*Passiflora* spp.) may bear simple, ternate or palmate leaves which all show remarkable similarity in the venation and other features of the leaves or leaflets. Similarly the leaves of *Forsythia* may range from simple to lobed, compound ternate and compound pinnate, but again the venation, marginal serrations and other features are similar throughout. Where a plant exhibits a

range of foliage of this kind, it evidently means that factors affecting leaf development at the apex have also been gradually changing and the largest and most complex leaf forms usually occur about midway along the shoot as evidences of maximum vegetative vigour. It is very difficult to identify the particular factors in operation or to determine the way in which they function, but there are a few cases where particular forms of leaves do appear to be associated with some definite factor, either in the plant itself or in its environment. In an earlier chapter (Chapter VI) the life cycle of an annual plant was discussed, and it was shown that with increasing leaf surface, the dry weight of the plant rises as organic matter accumulates. This is apparently associated with a change in the relative proportions of organic and inorganic food constituents reaching the growing point which finally induces the change from the vegetative to the reproductive phase. Associated with this change in the balance of food supplies the leaf form gradually alters. Very many examples of this might be cited, but the effect is seen very markedly in rosette plants, such as *Saxifraga* spp., where the vegetative leaves of the rosette are larger and different in shape from those borne on the elongated axis of the inflorescence. Amongst perennial plants, two striking examples of the same effect are holly (*Ilex Aquifolium* L.) where the leaves in the neighbourhood of the flowers often lose the spiny characters of the leaves of vegetative parts, and ivy (*Hedera Helix* L.) where the leaves on reproductive shoots are relatively narrow and simple compared with the wider, palmately lobed leaves of vegetative parts. A case that may be noted in hedgerow or garden is that of various species of *Rubus* (bramble, raspberry, loganberry), where the leaves on vigorous vegetative shoots have five leaflets, whilst those at the end of the vegetative shoot or on flowering shoots have three leaflets.

In some plants with modified leaves, such as gorse (*Ulex europæus* L.), where the adult leaves are reduced to spines, the effect appears to be due to lack of sufficient water. Seedlings of gorse usually bear a pair of cotyledons followed by ternate and simple leaves, which show a gradual transition to the spines characteristic of adult parts (Fig. 44). In wet seasons it is not uncommon to find a shoot which has been producing spines reverting to the flattened, softer foliage of the seedling.

A difference in leaf shape due perhaps mainly to water supply may also be seen very commonly if leaves from the crown of a tree are compared with those from a shoot springing from the base of the tree and hence nearer to the water supply from the roots. Leaves from the latter are larger and often more lobed, e.g. mulberry (*Morus* spp.).

Lack of sufficient water supply is the determining factor in the type of vegetation in certain geographical regions. In the coastal *mâquis* of south-west Europe, in the chaparral of the Californian coast and in certain parts of S.W. Australia

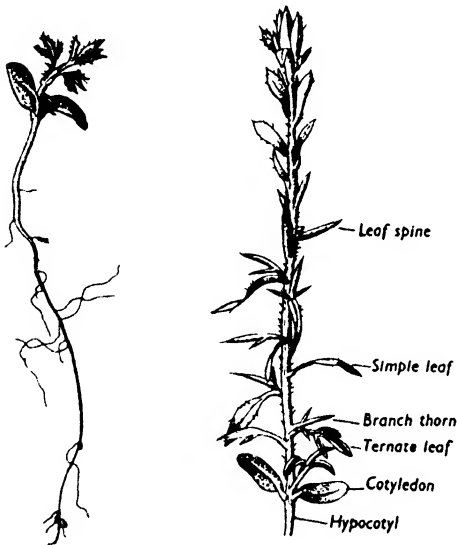


FIG. 44.—Gorse seedlings showing transitions from cotyledons, through simple and ternate leaves to spines ($\times \frac{1}{2}$).

and Cape Colony, the rainfall is confined to a short winter season, the rest of the year being very dry. The result of these climatic conditions is that the vast majority of the plants have tough, leathery leaves, which are sometimes small or spine-like. In this type of vegetation there occur also a certain proportion of succulent plants, in some of which the stem is thick and fleshy, and the leaves reduced to spines. This type of vegetation is spoken of as xerophytic (see Chapter XXI) and is in contrast to the vegeta-

tion characteristic of areas of abundant rainfall where the plants tend to have larger, thinner leaves.

Comparison of plants grown in light and darkness shows that some degree of light is essential for the full development of leaves. In nature it is very difficult to isolate the effects of insufficiency of light from other factors operating at the same time. Comparison suggests, however, that the development of leaves submerged under water has a similar effect to lack of sufficient light, and that both have the effect of reducing that type of growth which forms the expansion of the

lamina. Amongst dicotyledons a simple example is the water starwort (*Callitriche stagnalis* Scop.). At the surface of the water the plant produces a rosette of ovate leaves, whilst those on submerged parts are narrower and separated by longer internodes. In the water buttercup (*Ranunculus heterophyllus* Weber.), the floating leaves are palmately lobed, whilst under water the wing growth between the leaves is so little developed that the leaf is dissected into a number of linear segments, consisting of little beyond the vein system. Amongst monocotyledons, water plantain (*Alisma Plantago-aquatica* L.) and water arrowhead (*Sagittaria sagittifolia* L.) begin to grow in the spring, when the water level of the ponds is usually high, with the result that the first leaves fail to reach the surface. Such totally submerged leaves are long and strap-shaped. As soon as leaves begin to reach the surface of the water, whilst still capable of growth, a distinct lamina is formed by increased surface growth between the veins, producing the wide ovate lamina of *Alisma* or the characteristic arrow-head leaf of *Sagittaria* (Fig. 45). If plants of either of these two monocotyledons are grown in darkness, only the linear type of leaf is produced either under water or in air.

Inspection of leaves on horizontal shoots often suggests that gravity may have some effect on leaf growth, probably by influencing the movement of food supplies in the shoot. In almost any plant which bears its leaves in alternating pairs (i.e. decussate arrangement), such as horse chestnut (*Æsculus Hippocastanum* L.) or sycamore (*Acer Pseudoplatanus* L.), it will be found that on a horizontal shoot the two leaves of a horizontally placed pair are equal in size, whilst of the vertically placed pair the leaf directed downwards is distinctly larger. In such instances the effect is so consistent that there seems little doubt that the leaf primordium on the lower side has been better nourished. Cases of asymmetry of individual leaves also occur, but in this the results are so variable from one species to another and the possible effects of gravity so impossible to isolate from other factors, such as the arrangement of the leaves on the stem and the manner of folding of leaves in the bud, that further examination of individual cases is necessary before anything of a general nature may be said.

This short survey of a few cases where a suggestion can be made as to a factor influencing leaf development merely

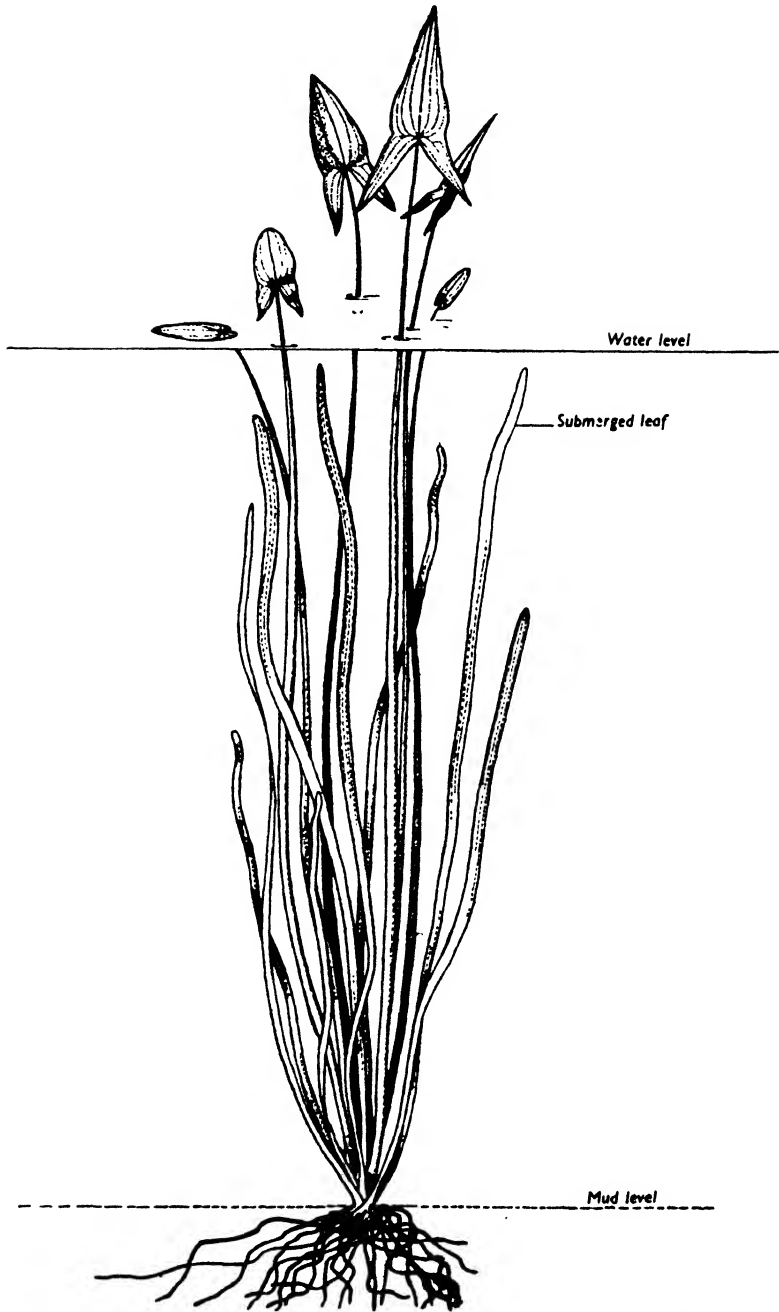


FIG. 45.—*Sagittaria*, showing forms of submerged and aerial leaves ($\times \frac{1}{4}$).

serves to show how little is known on this subject at present. Very many instances might be given of striking forms and variations of leaf shape, for which no explanation seems at all adequate. For example in the New Zealand flora many of the plants have a "juvenile" type of foliage which may persist for many years and which is so distinct from that borne by the plant later on, that it is often difficult to realise that the two belong to the same species. The "juvenile" condition lasts too long to be explained satisfactorily as due to changed nutrition as more leaves develop, and is not associated with the change to the reproductive phase. Such cases must simply remain on record until more facts have been accumulated about leaf development.

CHAPTER XIII.

THE ARRANGEMENT OF LEAVES ON THE STEM.

THE leaves of any plant are not only characteristic in form but also in the manner of their arrangement upon the stem. The system of leaf arrangement in a plant is spoken of as its phyllotaxis, and so characteristic are the various systems exhibited by different plants that they have given rise to much discussion.

The plan of leaf arrangement can be more easily followed in the dicotyledons than in the monocotyledons owing to the narrower leaf insertions, which enable the relative positions of successive leaves to be more readily determined. In the simplest case, as in the pea or bean, a single leaf is borne at each node and all the leaves are seen to lie in two vertical rows or orthostichies. Since successive leaves are inserted on opposite sides of the axis, this arrangement is usually spoken of as alternate. More frequently the leaves, though still inserted singly at the nodes, fall into more than two vertical rows and their insertions appear to lie along a spiral round the stem. Such leaves have been formed in succession in the bud of a growing plant so that the spiral system is not likely to conform to any perfect geometrical pattern, but usually it is sufficiently definite to show that a particular spiral arrangement is characteristic of any one species of plant. The spiral may be described in terms of the fraction of the circumference of the axis which separates one leaf from its nearest neighbour above or below in the spiral. In practice this is readily determined if a piece of thread is attached to one leaf and then carried spirally round the stem, passing successive leaf insertions by the shortest route, until a leaf is reached which lies vertically above or below the starting-point. In a specific case the leaf vertically above the starting-point may be found to be the sixth, when also the thread will be found to have travelled twice round the stem. Evidently the distance

covered by the thread between each of these six leaves will be one-fifth of the double circumference of the stem or two-fifths of the single circumference, so that the phyllotaxis is described as a $\frac{2}{5}$ system, $\frac{2}{5}$ being the fraction of the circumference which separates successive leaves. In this notation the alternate system would be $\frac{1}{2}$, and experience shows that the commonly occurring systems of spiral phyllotaxis fall into the series $\frac{1}{2}$, $\frac{1}{3}$, $\frac{2}{5}$, $\frac{3}{8}$, $\frac{5}{13}$. . . , known to the mathematicians as the Fibonacci series. The numerator and denominator of each succeeding fraction in this series may be obtained by adding together the numerators and denominators respectively of the two previous fractions in the series. In the $\frac{2}{5}$ system we have five vertical rows of leaves or orthostichies, a number given by the denominator of the fraction, whilst the numerator indicates the number of times that the stem is encircled before a leaf is encountered in the same orthostichy as the one from which a start was made.

In each succeeding fraction of the series the number of orthostichies rises and also the number of times that the stem is encircled before another leaf is found in the same vertical row. As the series is followed into the higher fractions, $\frac{5}{13}$, $\frac{8}{21}$, $\frac{13}{34}$. . . etc., these numbers rise very rapidly, and clearly the vertical rows will become more and more difficult to recognise until it becomes impossible in practice to find a leaf which one can be sure lies vertically above another. In fact the whole series tends towards an ideal arrangement in which no leaf would lie exactly on the same vertical line as any other. It is possibly the realisation of this fact that has led botanists to suggest that the appearance of the Fibonacci series in phyllotaxis systems is explained by a tendency to divergences between successive leaf insertions which will so place the leaves that the lowest will be least shaded from the light by the upper ones. It is difficult, however, to see how leaf arrangement, which is determined in the bud as each new primordium arises at the growing point, can be governed by the ultimate distribution of the leaf surface in the light. It seems more reasonable to consider the factors operating during development, when the significance of another property of the Fibonacci series becomes apparent.

The values of the fractions in the series all lie between $\frac{1}{2}$ and $\frac{1}{3}$. In the alternate or $\frac{1}{2}$ system, each succeeding leaf

arises as far as possible from the preceding one, and in the $\frac{1}{3}$ system they arise as close to one another as $\frac{1}{3}$ of the circumference. Successive leaves in a spiral system very rarely arise closer together than this, and it may perhaps be significant that $\frac{1}{3}$ phyllotaxis is comparatively rare in dicotyledons. It occurs in the bud of *Alnus* and in some flower buds, but apparently a leaf primordium or a floral part does not frequently arise as near to its immediate predecessor as $\frac{1}{3}$ of the circumference of the growing apex. This is understandable since each primordium is evidently growing most vigorously, usually faster than the apex which it soon overarches, consequently its demands upon the food supplies may make it difficult for another similar centre of activity to arise in its immediate vicinity.

Each leaf is an emergence on the side of the growing shoot apex, therefore the natural place for the next is on the opposite side of the shoot. In the dicotyledon embryo the first two leaves are thus arranged opposite one another as two cotyledons. When growth is resumed on germination of the seed, new leaves continue to succeed each other and to arise at opposite sides of the apex. The simplest expression of this is alternate phyllotaxis, when the associated fact also emerges that when two successive leaves arise actually opposite to one another, only two are present at the apex at the same time. A third primordium will only arise when the one vertically below it has passed into the next stage of its development and the extension of an internode will soon carry it out of the actual region of the growing apex.

Frequently, however, with increasing food supplies the growing point grows larger, whilst the leaf primordia, on the other hand, soon attain their maximum size. It then becomes possible for more than two leaves to be growing at the apex simultaneously when each leaf cannot be exactly opposite its predecessor. Rarely this may result in a $\frac{1}{3}$ phyllotaxis, but a more stable system in the dicotyledons is that in which two successive primordia are at least $\frac{2}{3}$ of the circumference from one another, in which case five may be growing simultaneously at the apex without any two falling on the same orthostichy.

Thus in the $\frac{2}{3}$ phyllotaxis system it is usually found that there are five young leaf primordia crowded at the apex without internodal extension between them, whilst in the $\frac{1}{3}$ system there are usually eight. Beneath the actual apex there are other leaves

which are still growing, and with internodes in varying stages of extension between them, and the number of these is related to the number at the apex. In the $\frac{1}{2}$ type, there are usually two internodes which are at an early stage of extension and two or four which have not yet ceased to extend. In the $\frac{2}{5}$ type, corresponding with the five primordia at the apex, there are usually five internodes in the early stages of extension and five with longer internodes which have not yet ceased to extend. In the $\frac{3}{8}$ type usually eight internodes in an early stage of extension and eight longer internodes still extending.

The degree of complexity of a spiral phyllotaxis system will depend upon the relative size of apex and primordium and the consequent number of primordia which can grow at the apex simultaneously. Usually the system reaches an equilibrium when the fraction of the circumference separating successive primordia can be expressed as a term of the Fibonacci series. In phyllotaxis, however, this series of fractions is employed only as a simple nomenclature for definite systems of leaf arrangement, which are determined in the bud by the conditions of symmetry involved in the growth of a number of leaf primordia simultaneously at the apex. This number is definite in any particular system and the emergence of a new primordium at the apex is associated with the separation of an older one from the bud ; both these primordia ultimately appear as two leaves on the same vertical row.

Such spiral systems of phyllotaxis are very common in plants, but they are not the only systems which may arise as the result of the successive appearance of primordia at the growing apex. The commonest variation is due to the development of a difference in the time interval between the appearance of successive primordia. Frequently, following the two cotyledons, the next two primordia arise nearly simultaneously and opposite one another and then after a longer interval of time two more arise again nearly simultaneously. Naturally the insertion of successive pairs, viewed from above, lies in planes at right angles to one another and such a method of development results in the opposite and decussate system of phyllotaxis, which characterises certain families of flowering plants, as for example the dead nettle family (*Labiatae*). In this system two pairs of young primordia occupy the growing apex at the same time, the members of

successive pairs alternating. As the growing point increases in size, a similar arrangement may hold with three or more primordia arising almost simultaneously. Such "whorled" systems are relatively common in dicotyledons and frequently show a variation in the number of members in the whorl as one passes up the shoot; often the whorls at the base of the plant have few members, whilst in the more vigorous part of the shoot higher up the members are more numerous. The members of successive whorls always alternate and are superposed above the members of the whorl next but one below.

In the monocotyledons, the tendency of the primordium to have a much wider insertion, which often encircles the stem, makes the phyllotaxis more difficult to study in the more complex systems. The ring-like insertion necessitates that, as primordia arise at the apex, they will do so in succession above one another and also inside one another. Apart from this the same factors apply as in the dicotyledons, for each primordium has a definite point of origin and this tends to arise as far as possible from the point of origin of the preceding primordium. Thus the spiral systems fall into the Fibonacci series and in whorled systems, which are found for instance in some species of *Lilium*, the members of successive whorls alternate. A fact that is of interest, however, is that $\frac{1}{3}$ phyllotaxis, rare in dicotyledons, is relatively common in monocotyledons and is the characteristic phyllotaxis of the big family of the sedges (Cyperaceæ). As in the dicotyledons relatively complex spiral systems may occur and this is found correlated with a large number of primordia, which are growing simultaneously at the apex of the shoot. The primordia expand outwards in succession as each new one is formed at the apex within the bud.

It is clear from this short discussion that if the primordia, which are forming at the apex, are considered as competing centres of growth, then the position of origin of a new one will be influenced by any others which are still growing at the apex, and this will have the effect of determining the types of spacing actually observed. In the spiral systems of phyllotaxis, as for instance in the $\frac{2}{5}$, when more than two primordia are growing at the apex at the same time and when two successive primordia are never found closer together than $\frac{1}{3}$ of the circumference, the actual positions occupied by the primordia are probably

determined by conditions of symmetry, as regards food supplies, etc. Successive primordia arise in these spiral systems after approximately equal intervals of time, termed plastochrones, and in the $\frac{2}{5}$ system it will be noted (Fig. 46) that each primordium is three plastochrones removed from its one neighbour, two from the other. All leaves, therefore, are developing under equivalent conditions, and this will be found to be true for all the spiral systems denoted by the Fibonacci fractions and may go far to explain the frequency of occurrence

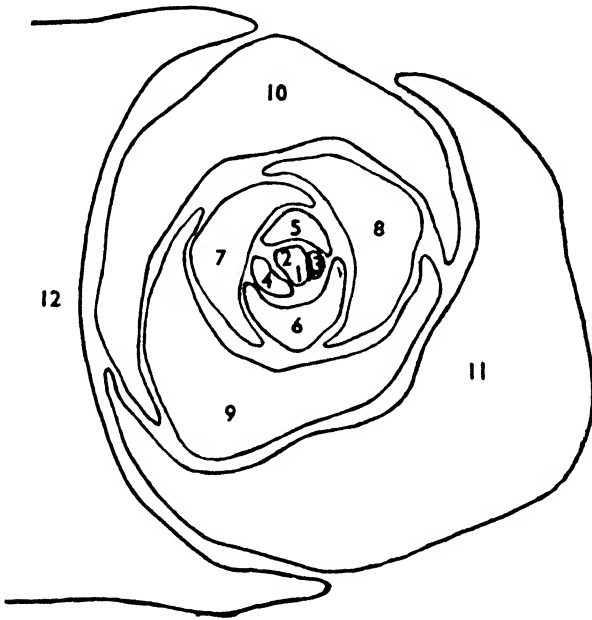


FIG. 46.—Diagram of a transverse section of a vegetable bud of *Ribes* with $\frac{2}{5}$ phyllotaxis ($\times 50$). Successive primordia are numbered.

of such spiral systems in plants. On the other hand, for any individual leaf the conditions under which it develops are not quite symmetrical if its neighbours exert any influence upon its growth. The individual leaf frequently is asymmetrical, but it is equally probable that this is caused by its own inherent growth as by competition with its neighbours. In all spiral systems the leaves arise singly at the growing apex, and this must mean that the apical growth itself has been asymmetric, in which case it is natural that its continuation in the leaf primordium separated from the apex should continue to be asymmetric also.

CHAPTER XIV.

AN INTRODUCTION TO THE MICROSCOPE.

OUR study of the shoot has so far been carried out by direct examination with the naked eye or occasionally with the aid of a magnifying glass or lens. However, even with this assistance the examination of such an object as the bud of the brussels sprout leaves one with a sense of incompleteness. In the dissection one leaf is removed after another until these become difficult to handle and so small that even their shape is not clear. Down to the limit of one's vision more leaves are seen, and the minuteness of the last that it is possible to remove leaves the impression that still smaller ones are probably present. For the last stages of such a dissection the use of a simple lens is of great assistance, as by this means a magnification of about ten times may be obtained, but even this is insufficient to give any detail of the actual growing point itself.

If one looks at some object, such as a tree, in the distance it appears small, but the nearer one approaches to it, the larger does it appear to be. If one goes nearer still, it becomes impossible to see the whole of the tree at once, but at the same time details of its surface may be examined, such as the texture of its bark and leaves. As this process of looking at the surface at closer and closer range continues, so does the apparent size and visible detail continue to increase, though with continued reduction of the field of vision, until the limit of clear vision is reached at a range of about 10 inches from the object. Within this distance the eye can no longer focus the rays so as to form a clear image on the retina. If it were possible to put the object still closer to the eye and still to see it clearly, it would be still further magnified, and the use of a simple lens has in essentials the property of making this possible.

When rays of light pass through a lens, they are all deflected from their original course except those which pass through the

optical centre of the lens. In the case of a bi-convex lens, rays from a distance, "parallel rays," on passing through the lens all converge to a point, the focus. By using a construction based on these standard rays, one is able to ascertain the position of the image which would be formed with the object in various positions relative to the lens. If the object is beyond the focal distance from a convex lens, an inverted, magnified image is formed at the other side of the lens and such an image is described as "real" as it can be received on a screen placed in that position. If, however, the object is placed between the lens and its focus and the same method of construction is employed, it is clear that the rays do not converge to a point at the other side of the lens and there is no position where a real image can be received on a screen. If the construction lines are projected back on the same side of the lens as the object, they do converge to a point, and if the object is viewed through the lens, the rays appear to reach the eye from this point, giving the impression of a magnified, but uninverted image. If the object were viewed at the same range without the lens no clear image would be formed, but the lens used in this way has the effect of making the rays appear to diverge from a greater distance and the eye can then perceive a clear and magnified image. An image produced by a lens in this way cannot be received on a screen and consequently is described as "virtual."

The compound microscope is a complex system of lenses, but for simplicity we may consider it as consisting of two convex lenses, one of which in the actual instrument is represented by the objective system (nearest the object) and the other by the ocular system or eye-piece (nearest the eye). When the lenses are correctly focused, the object on the stage of the microscope lies beyond the focal length of the objective lens. These distances are so arranged that the real image is formed in the ocular of the microscope (a tube with a lens at either end) at the level of a ring-like diaphragm. (The lens at the lower end of the ocular is actually part of the objective system and helps to bring the image to a sharp focus in the required position.) This image is inverted and magnified, and, being a real image, it is now possible to magnify it further by using the lens at the upper end of the ocular as an ordinary magnifying glass. Thus the inverted image from the objective

is seen through the eye-piece, as a still further magnified, virtual image which is still inverted (Fig. 47). This double magnification of the original object may be demonstrated very simply by a method we owe to Professor R. H. Buller. Draw a small D in black ink on a glass slide and place this in focus on the stage of the microscope. On a disc of transparent paper draw an arrow. Unscrew the upper or eye-piece lens from the ocular and place the paper disc so that it rests on the diaphragm in the ocular. Slip the ocular back into the draw tube of the microscope, with a slip of paper between ocular and tube to prevent the former slipping too

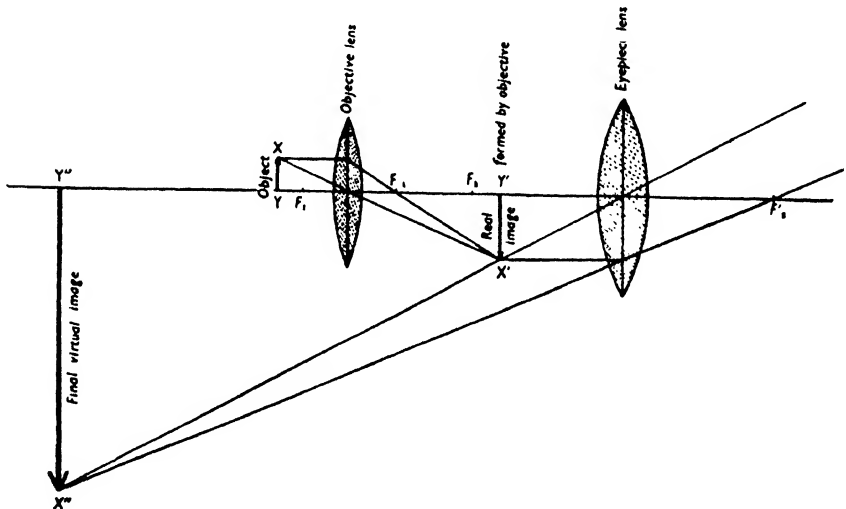


FIG. 47.—Simplified diagram of the optical system of the microscope.

far down the tube (it is normally prevented from slipping down by the rim of the eye-piece lens). On looking down the tube it will be seen that the arrow and the real image of the D are both visible on the paper disc. The D is of course inverted and magnified. Screw the eye-piece lens back into position and look down the microscope. The arrow and the D are now seen to be magnified but not inverted by this lens, showing that either an object (the arrow) or a real image may be magnified by the eye-piece lens. The letter on the slide has been magnified twice and the total magnification is the product of that by the objective system and that by the eye-piece lens.

In our morphological work external features were being examined, so that the object was placed in a good light and seen by virtue of the light reflected from the surface. Minute surface features may also be examined through the microscope, but it is then necessary to throw a very bright light on to the object as a good deal of the light is reflected away and does not pass into the microscope. More often objects for microscope examination are cut into very thin sections, or otherwise rendered transparent, and are then inspected by transmitted light. In order to have this sufficiently concentrated, a movable mirror is present beneath the stage to reflect the light up the tube. This is plane on one side and concave on the other, but for most purposes the plane side is used in conjunction with a sub-stage lens system to act as a condenser. For the best results the light should be made as bright as possible by adjusting the mirror and condenser and subsequently cut down to the desired brightness by partially closing the iris diaphragm, which is fitted just below the condenser lens. (Details concerning the manipulation of the instrument are usually provided with the microscope.)

As the object is now viewed as a virtual, enlarged image, it becomes more difficult to make such observations upon its nature and consistency as were possible when its size permitted that it could be seen and touched at the same time. Some useful evidence may, however, be obtained by the behaviour of light as it passes through the object. This may be illustrated by examining the appearance of globules of oil in a watery medium and comparing this with that of bubbles of air also in a watery medium. If a little olive oil is shaken up with water to which a small amount of caustic potash has been added, a suspension of oil droplets is obtained. A small drop of this is placed on a glass slide and then a cover-glass is dropped on in such a manner as to entrap a few bubbles of air between it and the slide. Examined under the microscope the oil globule has a dark outline and a bright centre, and as the focus is changed it is seen that the brightness of the centre varies, appearing smaller and at its brightest at a relatively high focus. The air bubble, on the other hand, appears similar at first with a dark outline and bright centre, but the effect on focusing is the reverse of that seen with the oil globule, for the smallest and brightest centre is obtained by focusing down.

These different light effects are due to the relative refractive indices of the inclusions as compared with the medium. The oil has a higher refractive index than the water, and thus light rays passing from water into oil are bent towards the normal (the ray which strikes the surface at right angles at the same point and passes through undeviated), and this results in the concentration of the rays at a relatively high focus. In the case of light passing from the water into the less dense air, the rays are bent away from the normal and consequently the greatest concentration of rays is at a low focus. Thus the observation of the behaviour of light enables one to compare the refractive index of an object with that of the medium in which it is mounted, and this comparison is often of great assistance in determining the general nature of the object examined.

A further difficulty in the study of objects through the microscope is that we cannot have any appreciable depth sharply focused at the same time. We have the alternatives of looking at a flat surface by reflected light or a transparent object by transmitted light. In the latter case, however, only a very thin layer of the object is seen sharply focussed at any one time. By focusing up and down a number of "optical sections" may be studied in a transparent object and, if a series of these are put together in our minds, it is possible to construct a mental picture of how the solid object is built up. Obviously this may be no easy task, and the outstanding difficulty of microscopic work, of which the beginner needs constant reminder, is the continual need for this mental reconstruction of objects in the solid from the series of enlarged, virtual images, projected in one plane, which are the evidence of structure presented to the eye. Drawings from the microscope tend to emphasise the impression that the object studied has only two dimensions and therefore it is very valuable at the outset of microscopic work to complete one's mental picture of the enlarged view of the object studied by combining these impressions in a three-dimensional model, constructed in plasticine or other suitable material. When this task is undertaken, many observers will realise for the first time how incomplete has been their appreciation of the structure of the object as revealed to them by their observations through the microscope.

When we attempt to study an object by transmitted light, the preparation of a suitable mount of the material for examination and the correct adjustment of the light are important details of technique that are only mastered with experience. The object may be so dense that too little light can pass through it for any detail to be observed, and the beginner always has to learn that too much material on the microscope slide handicaps observation. On the other hand, the material may be suitably transparent, but if too much light is passed through it the eye may be unable to detect the slight differences in light intensity produced by much of the detail in the virtual image. This can usually be overcome by adjustment of the iris diaphragm.

The Cell.

Before attempting to use the microscope to elucidate the structure of the growing region of a shoot, a preliminary examination of simpler objects is advisable. A very suitable object for a first study under the microscope is the flesh of the snowberry (*Symphoricarpos racemosus* Mich. var. *laevigatus* Fernald)*. When the skin of the berry is broken, a small quantity of the bulky flesh may easily be removed and placed on a glass slide for examination. If this is examined under the microscope in the light transmitted from below by the mirror very little detail can be made out. Irregular globules are seen, each with a black outline, whilst in the centre a brighter spot suggests that this region of each globule condenses the light during its passage. The black outline means that no light is reaching the eye from the surface of the globules, which obviously is accounted for by light rays from the mirror beneath the stage being bent in such a way at the surface of the globules that they do not continue to travel in such a direction as to reach the eye. Such a sharp change in the path of light takes place when the medium in which it is travelling undergoes a sudden change in refractivity, as for example, when light passes from air to water. The angle which a straight stick appears to make where it enters water from air is a well-known illustration.

This point is all important for our interpretations of microscopic observations and may be illustrated by the following experiment. A large test tube is filled with water,

* *Symphoricarpos albus* (L.) S. F. Blake

and a small test tube one-third filled with water inverted into it. Half the water is then poured out of the large tube, which is now examined when standing in a flat-sided glass vessel containing water. In the lower part the inner tube is very transparent and clearly seen, as the light passes through successive layers of glass and water, and the transparency of the whole system is due to the fact that the refractive index of glass is near to that of water, and hence the light rays are not appreciably bent or reflected at the surface of separation of the different media. At a higher level the inner tube containing air is surrounded by the outer tube containing water. A proportion of the light, on passing from the denser water and glass to the air, is totally reflected and does not enter the inner tube at all, so that viewed from the far side, the inner tube has a somewhat indistinct, black outline and appears less transparent. At a still higher level, where the inner tube containing air is surrounded by the outer tube also containing air, the whole system is again more transparent but not quite so distinct as at the base, as the glass and air differ more in their refractive indices than glass and water.

A second experiment is to compare the appearance of a glass rod in water and in pure glycerine. The rod appears more transparent in glycerine than in water as the refractive index of glass is nearer to that of glycerine.

Returning now to the flesh from the snowberry under the microscope, the black outline suggests a change in the refractive index of the medium at the surface of the object, whilst the brightness at the centre of the globules suggests that the material within them is denser than the air in which they are being examined. We know that 90 per cent. or over of such berries consists of water, so that the probability is that these globules are full of water. If we add water to the material on the slide (or preferably make a fresh mount, placing the flesh in a drop of water) so that there is water outside as well as inside the globules, we find that the whole appearance is now much more transparent and the outline of the globules can be seen very sharply. Thus this examination of the virtual image of the cells has given us grounds for thinking that their contents are aqueous.

For further examination a cover-glass should be laid gently on the preparation so as to bring the cells more nearly

into one plane and prevent reflection from the irregular surface. When clearly focused we now find that the whole of the fleshy material mounted consists of globules of various shapes, which are frequently more or less rounded. The outer surface of each globule may be seen as a sharp line, which means that it differs more in refractive index from the watery medium without and is perhaps therefore a more definite non-aqueous substance than the rest of the globule within (Fig. 48a). As we focus up and down on this outline, using the high-power objective of the microscope, the exact limits of any globule under observation alter, which obviously means that this layer has a curving outline above and below. Crossing the globules sharp lines come into the field of vision which seem to have a refractive index similar to that of the periphery of the globules. The lines are evidently some feature of the peripheral layer and the natural interpretation is that they are wrinkles in an almost transparent, solid, jelly-like wall, which surrounds each globule. When a compound lens system was first used to examine a plant, the first point established was that the plant was constructed of a number of cavities enclosed by more refractive walls, and its structure was consequently described as cellular, using the term in the same sense as it is applied to a honeycomb. The units which constitute the loose flesh of the snowberry are cells, and, with very few exceptions, we shall find that this is true of the construction of plants.

The external bounding layer is known as the cell wall, and when plant tissues are pulped and all their readily removable contents removed by treatment with alkali, the walls of the cells remain in the pulp and cling together, as it dries, into a sufficiently solid mass after suitable treatment to give a paper. If a fragment of paper is treated with 70 per cent. sulphuric acid or concentrated zinc chloride (2 parts zinc chloride to 1 part of water by weight), it becomes much swollen, and on addition of a drop of iodine solution (iodine 1 part, potassium iodide 1 part, water 100 parts by weight) the material turns blue, a reaction which is characteristic of cellulose. With care this test may be applied to the cells of the snowberry. Mount some of the material in iodine and then draw off most of this aqueous liquid by absorbing it with a piece of blotting paper. Then add a small drop of 70 per cent. sulphuric acid or of zinc chloride rather to one side of the

material and put on a cover-glass. Where there is much acid some of the walls will swell too much and soon dissolve, but

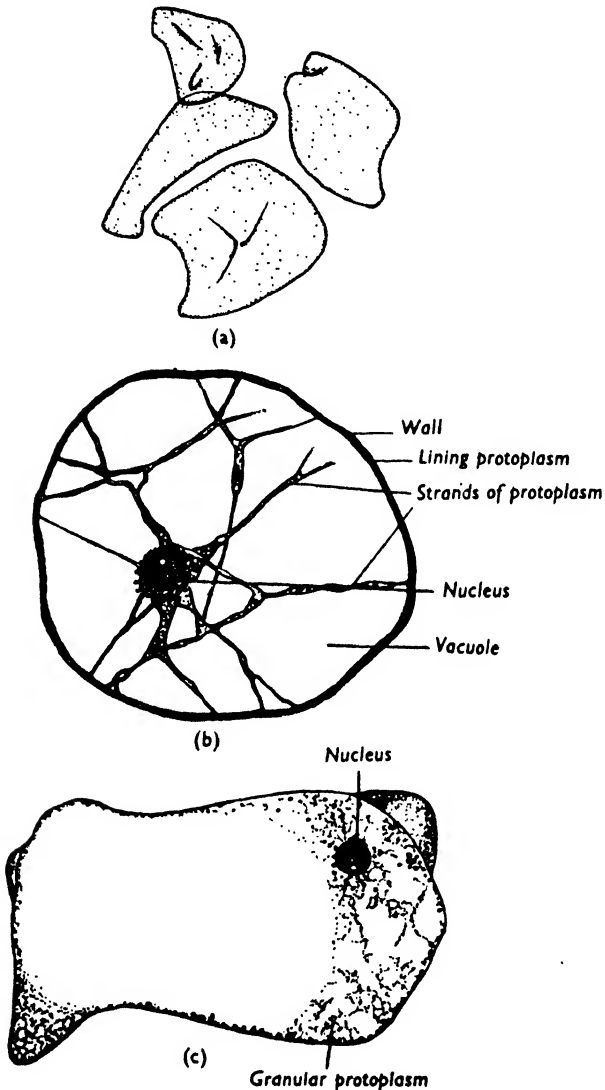


FIG. 48.—Cells of snowberry, (a) mounted in air ($\times 70$), (b) mounted in water ($\times 250$), (c) mounted in iodine solution ($\times 250$).

where there is a suitable concentration of acid some walls are sure to be swollen to the right stage, and these will give a blue colour with the iodine, which they have already ab-

sorbed. Cells which are insufficiently swollen remain yellowish in colour.

When cells mounted in water are examined under the microscope, it is not possible to see many details of structure within the cell. Some indication of fine granulation might be noticed when the high-power objective was used, but the appearance of this is not very constant, and indeed in some cells from berries in good condition, the minute granules often may be seen to be moving, as though carried along by some invisible stream which is travelling in one direction in one part of the cell and in a different direction in another part. As the focus is altered, somewhere within the cell a more highly refractive spherical or oval body may be seen. The more refractive appearance of this body, which makes it visible, suggests also that, if aqueous in nature, it is denser than the rest of the contents of the cell, but apart from the fact that one or more still more highly refractive dots may be seen within it, no further detail is visible (Fig. 48*b*).

If such cells are examined mounted in iodine solution, these colourless internal structures become much more evident and are stained yellow; the granulation appears denser and is unevenly distributed. The denser body previously observed is also more obvious in iodine, which stains it deeply (Fig. 48*c*). These observations on the contents of cells took the earlier scientists many years to elucidate. As soon as plants were examined with the microscope, the cell and cell wall were discovered (R. Hooke, 1665), but the significance of the transparent "slime" which may even be seen in movement in the living cell and which becomes denser and more coarsely granular on treatment with iodine, was not realised until von Mohl drew attention to it in 1846, and called it protoplasm. Since then attention has shifted more and more from cell wall to protoplasm, which is the substance which Huxley described in 1868 as "the physical basis of life." Chemical analyses tell us that the walls of plant cells are made of cellulose, a carbohydrate, but that the living protoplasm always contains a still more complex class of chemical compound, the proteins (Chapter XVIII).

Iodine precipitates proteins, and the change in optical appearance that we have noted is largely due to the reaction of the iodine with the proteins which form the basis of

protoplasm. A special part of the protoplasm is the denser, oval or spherical body, which stained more deeply with iodine and is universally present in living plant cells and known as the nucleus. The nucleus was discovered by Robert Brown in 1831, a little over a hundred years ago. The general granular material and the nucleus are both included under the term protoplasm, the two distinct regions of which are distinguished as the cell protoplasm or cytoplasm and the nucleus.

The observations made upon the living cells and upon the iodine stained cells enable us to draw a further conclusion. The granular protoplasm is sharply in focus near the wall when the edge of the wall is in focus; the granules are only visible over the centre region of the cell when either the back or front wall is nearly in focus. Furthermore, when movement of granules in the living cell is seen, the movement is not in a whole sheet, but in the form of distinct strands which often run to or from the nucleus. The appearance of strands suggests that the protoplasm is not continuous throughout the interior of the cell, whilst the other observation suggests that there is a peripheral sheet near the wall, so that we may tentatively conclude that there is a sheet of protoplasm just within the wall, in which the nucleus may lie, or this may be suspended more centrally in the cell by strands of cytoplasm which link up with the peripheral lining. In the rest of the cell cavity nothing can be seen, which indicates that the refractive index of this region is near that of the watery liquid in which the cells are mounted and therefore that the greater part of the cell cavity is filled with a watery liquid. This is known as the cell sap and the space in the protoplasm in which it is contained is the vacuole. When the nucleus is lying in the peripheral sheet of the protoplasm, usually one large vacuole occupies the whole of the central part of the cell, but when the nucleus is more centrally placed, as is often the case in rather younger cells, this may be associated with strands or sheets of cytoplasm extending from the peripheral sheet and between which numerous smaller vacuoles may be present.

In the berries of privet (*Ligustrum vulgare* L.) the flesh consists of cells loosely held together, very similar to those of the snowberry, but those immediately beneath the skin contain a red pigment dissolved in the sap. Mounted in water, the

red coloration seems to fill the cell completely ; in addition small granules are present which contain green pigment and are called chloroplasts (Fig. 49*a*). If the cells are mounted

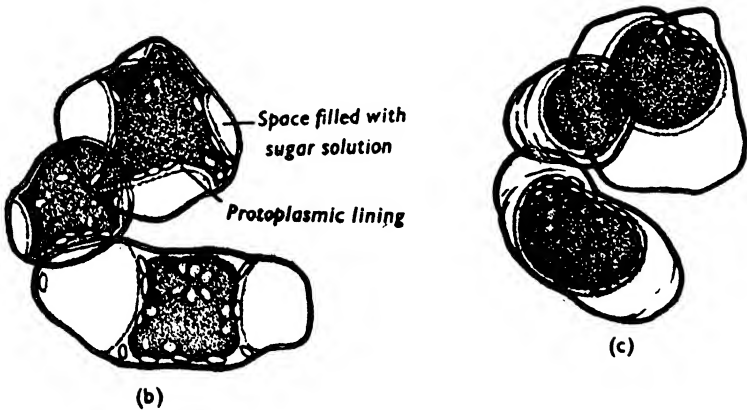
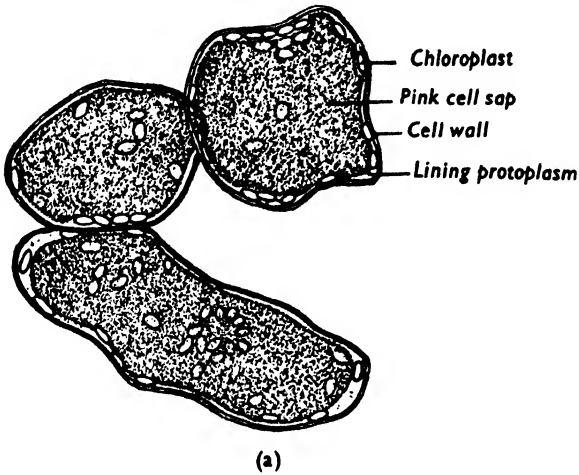


FIG. 49.—Cells of privet berry, (a) in water, (b) after a few minutes in 30 per cent. cane sugar solution, (c) after 10-15 minutes in sugar solution ($\times 450$).

in a 30 per cent. cane sugar solution, water is withdrawn from the cell vacuoles by the strong solution outside and at the same time the protoplasm, still containing the colouring matter

and the chloroplasts, gradually comes away from the cellulose wall and the resulting space is filled with the sugar solution which readily passes through the wall (Fig. 49*b* and *c*). Cells in this condition are said to be plasmolysed. It is now possible to see that the protoplasm must have formed a continuous sheet lining the cellulose wall, and evidently it is this layer which is responsible for the retention of the coloured sap. This protoplasmic layer is semi-permeable in that it allows water to pass whilst retaining within it certain substances in aqueous solution and not allowing other substances, as the sugar in the external solution, to pass in. Semi-permeability is only a property of protoplasm so long as it is living ; if the microscope slide is held for a few moments above a flame until the heat has killed the protoplasm, the red sap immediately escapes from the cells and is seen to accumulate at the edge of the cover-glass. As the protoplasts are killed, they contract and appear as small masses of quite irregular form.

The conclusions which have been drawn are so fundamental that they would not be justified if based solely on the few observations described, but it seems reasonable to profit by the experience of our predecessors and to accept tentatively at this stage their guidance in these matters. Our own direct observations have been limited to the interpretation of a series of virtual images in which no two cells look exactly alike ; the observations have also been made upon fresh material in which the condition of the material and the method of mounting give rise to different appearances, and further the appearance alters with every change in the focus and the degree and direction of lighting. All these points need to be borne in mind when attempting to arrive at an interpretation of the structures examined.

It is obvious that great care is necessary in making suitable mounts to examine under the microscope. In all cases only a very small amount of material should be placed on the slide, and this should be mounted in a small drop of liquid, just sufficient to run to the edge of the cover-glass all round, but not to allow it to float, when this is gently lowered from one side on to the material. Water is usually the best mountant for fresh material, 10 per cent. glycerine for material preserved in alcohol. It is most important that the slide and cover glass should be clean and no liquid must be allowed to wet the

surface of the objective lens, as this prevents the formation of a clear image.

The Size of the Cell.

When making observations and large drawings of structures examined under the microscope, it is easy to lose sight of their actual dimensions. It is very desirable that the magnification of drawings should be definitely on record, and a very simple method may be devised to give an indication of the actual sizes of small microscopic objects which cannot be directly measured. A piece of artificial silk is selected of suitable texture, and a measured distance of 1 centimetre is marked upon it by making two dots upon one definite thread of the silk. (Artificial silk is more suitable than materials of natural fibres owing to its greater uniformity.) The number of threads running in the same direction between the dots is counted with the aid of a lens. Owing to the woven structure it is simpler to count the threads which pass over the thread at right angles and then to double this number. The material is then examined in the same direction under the low-power field and the number of threads counted across the diameter of the field. In an actual example, 50 threads were present to the centimetre and 8 were seen in the low-power field. The diameter of the low-power field is then $\frac{8}{50}$ of a centimetre, or 1.6 mm. In a similar manner the actual dimensions of the high-power field may be obtained. A cell may then be examined under the most suitable power of the microscope, when it is usually possible to decide with a sufficient degree of accuracy how many times the cell could be fitted into the diameter of the field and thus its real dimensions may be calculated. The measurement in the same direction on the drawing may be divided by this value and thus the magnification determined and stated beside the drawing. If the same microscope is used each time, a record may be kept of the diameters of the two fields and the magnifications of all drawings may be calculated and recorded.

An Introduction to Cell Aggregates.

We have so far confined our attention to the "unit of construction," the cell. Judging by the number of cells

in the small speck of the snowberry flesh mounted for examination, there must be a very great number of such cells in the whole berry, and if the whole plant is similarly constructed the number must be almost innumerable. The greater part of the plant has a firmer texture than the flesh of the berry which must mean that the cells are more firmly attached together. The flesh of the berry is particularly favourable as a first object of study because the cells readily fall apart, and a little of the flesh, removed with a needle and mounted in water, shows us a number of separate, individual cells. Before we try to examine a plant built up of many such cells firmly fixed together, it is well to examine a series of simple green plants which illustrate steps in the aggregation of cells.

Occasionally when rain water accumulates in a water butt or other receptacle it develops a green colour. This may be due to the presence of very large numbers of a unicellular, microscopic plant known as *Chlamydomonas*. If a drop of this green water is examined on a slide under a cover-glass, a number of very minute, pear-shaped cells are seen to be moving about very rapidly, the more pointed end always travelling foremost. Each cell is so small that little detail can be seen under the low power, whilst under the high power the movement is so rapid that the cells move in and out of the field of vision very quickly. It frequently happens, however, that some cells may become caught in some way so that they cannot move out of the field, and in such a case it is possible to see that the whole cell appears green except for a very small pointed, anterior region. Towards this anterior end there is at one side a minute red spot, known as the eye spot. With very careful adjustment of the light it may occasionally be possible to see that at this end there are two very fine, colourless threads, one attached at each side (Fig. 50a). These flagella are very long, usually longer than the whole cell, and it is due to their lashing in the water, by a movement very similar to a swimming stroke, that the cells are propelled about in the water. Towards the wider end of the cell, focusing up and down will show that there is a denser, more refractive body contained in the cell; this is a protein body called a pyrenoid. If a drop of iodine is added to the water, the cells are killed at once and are consequently more convenient

to examine. The flagella are protoplasmic in nature and their yellow staining in iodine often renders them more visible, but they are particularly clearly seen if the cells are mounted in a gentian violet solution containing osmic acid (20 c.c. of an aqueous 0.1 per cent. gentian or crystal violet solution are mixed with 10 drops of 1 per cent. osmic acid shortly before use). The deep purple staining shows the flagella clearly, but darkens the cells too much for examination of other detail.

When conditions are suitable these minute organisms multiply extremely rapidly. Sometimes the cells lose their flagella (probably these are shed) and pass into a *Palmella* stage (Fig. 138), in which the contents of a cell divide into

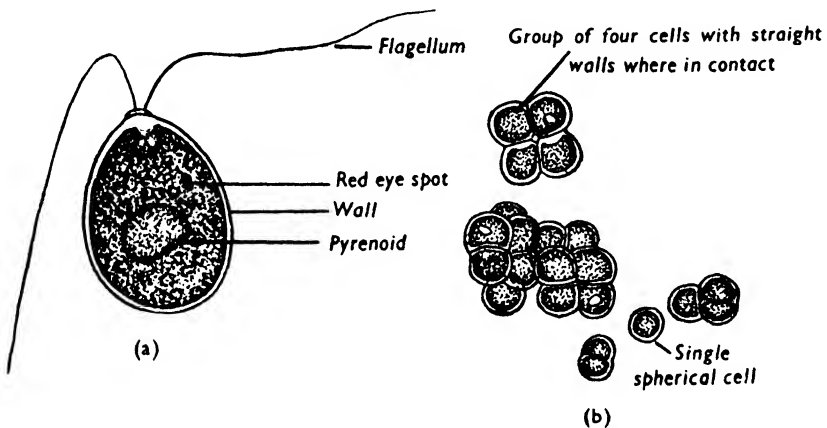


FIG. 50.—(a) *Chlamydomonas* cell ($\times 1000$), (b) cells of *Pleurococcus* ($\times 400$).

two parts, around each of which a new wall is formed. The walls then swell and form a mass of soft mucilage in which the two daughter cells remain embedded. Divisions of this kind are repeated frequently so that a gelatinous mass results. The individual cells in this are only held together by the surrounding jelly and at any time a cell may escape from the jelly, develop flagella and swim away as a motile individual. The cells may also multiply by another method in which the contents of the cells divide up into 2, 4 or more numerous parts, which round off from one another within the wall of the parent cell. At this stage the colourless wall of the parent cell is clearly seen as all the protoplasmic contents, which also contain the green colour, have been withdrawn

from it ; the cells also as a consequence lose their flagella. The wall of the parent cell soon becomes ruptured and the contained bodies are liberated into the surrounding water. Each one immediately swims away and presumably flagella were already formed at the time of its liberation. It differs from its parent only in its smaller size and in the fact that it has no cellulose wall and is simply a naked drop of protoplasm. The new cells swim about in this way for some time and afford good evidence of the fact that living protoplasm is the essential constituent of the living cell. After a time each daughter cell is able to form a wall, that must obviously be made by the protoplast, which might thus be regarded as analogous with a living inhabitant which builds a house round itself. In this plant each cell is a complete entity and can carry on all its processes of life quite independently of all other cells ; thus *Chlamydomonas* is an example of a strictly unicellular plant, in which this is particularly evident owing to the separation of the cells after division, owing to the motility of the cells.

Another simple green plant, *Pleurococcus*, is found very commonly on the bark of trees, where it may be present in such quantities as to give the whole surface of the trunk, especially on the windward side, a bright green appearance. If a little of this powder is mounted in water under a cover-glass and examined, it will be found to consist of small groups of 2, 4, or more cells, amongst which are a certain number of single cells (Fig. 50*b*). The latter are almost spherical, but when the cells are held together in a group, the outer walls are curved whilst the sides where the cells are in close contact are straight. Obviously the contents of a living, growing cell must press against the wall so that the whole structure becomes swollen up like a small balloon and so the single cell is spherical. However, when two cells are in close contact the free outer side is rounded, but where the contents of the two neighbouring cells are exerting equal pressure against one another, the sides remain straight. The natural inference is that, as the cells grow they divide and the two new cells, which are not motile as in *Chlamydomonas*, do not immediately fall apart. Details of division are not readily seen, but we may reconstruct the probable stages of the process. Presumably the protoplast divides first as in *Chlamydomonas* and each portion, now with-

drawn from the wall of the parent, secretes a new wall around itself. The new walls continue to be thickened by the protoplasts with which they are in contact and the new cells grow in size, whilst the outer common wall of the parent cell is stretched until it breaks. The new cells are then free to expand and will round off and separate from one another, so long as the cell walls are not held together by any cementing substance.

In many of these simple plants which occur in water or damp air and which are known as Algæ (Chapter I), there is found enveloping the single cells or groups of cells a layer of slimy mucilage which appears to have been formed by the cells. The cells of *Pleurococcus*, growing on the bark of trees, may become very dry at times, and if such mucilage is present it will tend to dry to a cement between two cells which are closely adpressed and so may delay the separation of the individual cells.

In another very common alga, *Spirogyra* (Fig. 51), found as a slimy mat suspended in the water of ponds, the cells always divide in the same plane and then remain attached together to form long threads or filaments of green cells. Every cell of a filament is similar in appearance and each one seems to be equally capable of growing and dividing. If the filament is broken or if a cell dies, the neighbouring cells seem to be completely unaffected and in fact each cell seems to be capable of living as an independent individual. It is thus difficult to know whether we should speak of the whole filament as an individual which grows in length, or as a colony of unicellular individuals which increase in number. The individual cells of the filament are relatively long and cylindrical and are characterised by the way in which the green pigment is held within the cell as a film, which has the form of one or more long, ribbon-like bands spirally arranged around the inside of the wall of the cell, much as a strip of green paper might be coiled within a glass tube. The clearness with which these details may be focused in a filament in water shows that the spiral green strip, the chloroplast or chromatophore, is suspended near to the wall in an aqueous medium. It is usually also possible to see the nucleus towards the centre of the cell, surrounded by a thin sheath of cytoplasm from which strands radiate to the layer of cytoplasm which lines the wall and in which the chloroplast

also lies. Our immediate object, however, is to consider the structural features that keep the cells attached. As in the previous cases, when the protoplast divides into two, each daughter protoplast secretes a new cell-wall and these new walls lie closely pressed together within the cylinder of the old wall of the parent cell. In this plant the new

cells show little or no increase in girth as they grow and thus the old wall is only stretched in length and is not disrupted immediately. At the same time a cement-like substance accumulates between the two new cellulose walls, as may

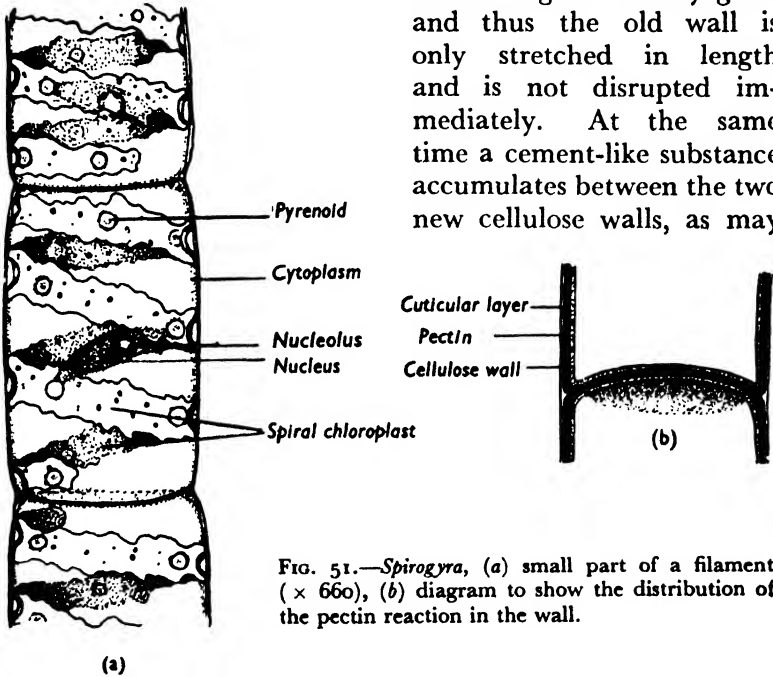


FIG. 51.—*Spirogyra*, (a) small part of a filament ($\times 660$), (b) diagram to show the distribution of the pectin reaction in the wall.

readily be shown by mounting the filament in a dilute aqueous solution of methylene blue or ruthenium red. These colouring matters are recognised reagents for staining a class of mucilaginous substances known as pectins. When one of these reagents is used on *Spirogyra*, the cross wall between two neighbouring cells of the filament is seen to have a median region which stains deeply; there is also some stain in the outer part of the free longitudinal walls, but very careful focusing shows that outside this again there is an extremely thin, refractive layer which is continuous along the whole filament and which is unstained. The filament of *Spirogyra* is thus seen to be constructed of a file of cells with separate cellulose walls which are held together at

their cross walls by a cement of pectin. A similar film also extends along the length of the filament and in addition the whole filament is surrounded by a thin layer of some other material which appears very refractive and may consequently be relatively rich in fatty substances. (For lack of a better term this is sometimes described as the "cuticular" layer.)

Pectic substances in contact with water tend to swell up into mucilage, and many of the aquatic algæ are surrounded by sheaths of such mucilage which gives them a slimy feeling to the touch, very noticeable in *Spirogyra*. The mucilage is continually diffusing into the water around and at the same time is being renewed by the activity of the living cells of the alga. When cell aggregates are growing exposed to the air, the pectins cannot diffuse away but dry to a sticky cement, which may eventually set very firmly indeed.

Mucilaginous pectic substances are always present in plant tissues and especially in fleshy fruits and roots. It is the presence of these substances in the fruits which accounts for the setting of jams and jellies without the addition of gelatine. They may be extracted from fresh fruits with water, as may be shown by crushing up the flesh of an apple and mixing it well with water. If this extract is separated from the pulp by straining through a cloth, and allowed to drip into a cylinder containing alcohol, a jelly-like coagulum of pectin settles out. It is known that a water-soluble pectin of this kind may change to a more insoluble substance, pectic acid, with the release of methyl alcohol and other alcohols. Like other acids, pectic acid readily forms salts, soluble salts with sodium, potassium, or ammonium, and insoluble salts with calcium. Thus if the apple is extracted with dilute caustic soda, a greater proportion of the pectin is extracted in the form of sodium pectate. After filtering, relatively insoluble pectic acid may be thrown out of solution by the addition of a mineral acid, or insoluble calcium pectate by the addition of a solution of calcium chloride. If a thin section of some fleshy root such as carrot or turnip is examined in water, it will be seen to consist of many cells which are firmly held together in a tissue. The individual cells evidently have the usual tendency to round off from one another, for they will be seen to be tearing apart to some extent at the angles, so forming intercellular spaces between them. If

thin slices of this kind are treated for 24 hours or longer in alcoholic hydrochloric acid (1 part of acid to 3 parts of alcohol), most of the pectins present are converted into pectic acid, but in the presence of the alcohol this remains insoluble. The sections are then washed in water and transferred to 0.5 per cent. ammonium oxalate when the pectic acid is converted into soluble ammonium pectate. The ammonium is added in the form of oxalate as this precipitates any calcium which may be present as insoluble calcium oxalate. If a section from ammonium oxalate is mounted on a slide in a drop of the solution and the cover-glass is gently tapped, the cells readily fall apart, each with its cellulose wall intact, showing that the tissue owed its original firmness of texture to the cementing together of the cells with a pectin cement known as the middle lamella. The presence of this *in situ* may also be demonstrated as follows. Warm a thin section of the tissue in dilute caustic soda, wash carefully in water and stain in dilute methylene blue or ruthenium red. Careful examination under high power now shows that the wall where two cells are in close contact consists of three layers, the unstained cellulose wall of each individual cell and the thin line of the stained middle lamella between them. Where cells have pulled apart from each other forming intercellular spaces, it is clear that these are bounded by the middle lamella so that the cells have separated from one another along this surface of contact.

After this brief review of certain simple cases of cell aggregates a return may be made to the study of the multicellular structure of the flowering plant, with the additional idea that the formation of such cell aggregates is the natural consequence of the growth of cells under conditions in which they do not fall apart from one another as they grow. The cell with its own separate cellulose envelope remains the natural unit of construction, but these are held together in a matrix which hardens and sets to a firm middle lamella composed mainly of salts of pectic acid. We must now consider the problem of how the growth of cells in such aggregates can lead to the form changes which we see proceeding at the growing point of a shoot.

CHAPTER XV.

THE SHOOT APEX.

IN dissections of the bud of the Brussels sprout and in other buds also, the last leaves which it is possible to remove are very small and very delicate and difficult to see clearly even with the use of a lens. If the central region of the bud is examined under the microscope by reflected light, little more is seen, and the apex, small as it may seem, is yet too massive to examine by transmitted light. It is therefore found necessary to employ a special technique for the further examination of the actual region where the leaves are being formed. The outlines of such a technique are only described here in order that the changes in the appearance of the material, due to the method, may be appreciated.

For this purpose it is convenient to select a plant in which it is feasible to cut a longitudinal section in a definite plane with regard to the leaves. Plants with leaves arranged in alternating pairs (decussate phyllotaxis), e.g. privet (*Ligustrum vulgare* L.), are suitable and have also the additional advantage of having a straight shoot. Tips of growing shoots in May or June are cut off about half an inch in length, and immersed under a "fixing fluid" after the outer leaves have been removed. The fixing fluid is a solution of various substances in carefully adjusted concentrations, which has the effect of killing the tissues very rapidly and with the minimum of distortion. After fixing, the tips are washed in water and then transferred by gradual stages of increasing concentrations into pure alcohol. In the same gradual way the alcohol is replaced by xylol, which is a solvent for paraffin wax. Wax is then dissolved in the xylol and the latter gradually evaporated off at a temperature slightly above the melting-point of the wax. When the buds are thoroughly impregnated with

the melted wax, this is cooled suddenly and thus the buds are embedded in a block of solid wax. The block may be cut into sections of desired thickness by the use of a special instrument, the microtome. The wax ribbons containing the sections of the buds are mounted on slides, to which they are fixed by means of albumen cement. The wax is dissolved away in xylol, the xylol is replaced by alcohol of decreasing concentrations and the sections are then stained in suitable dyes which are either in alcoholic or aqueous solution. Once more the sections are taken through the alcohols so as to remove all traces of water, and then into xylol as this is also a solvent for Canada balsam, the substance in which the preparations are finally mounted. This balsam sets hard and is a suitable mounting medium as it has a refractive index similar to glass. In this way permanent sections are prepared in any desired direction and of a known thickness. In the case of a longitudinal series few of the sections will be sufficiently median for the present study.

The Shoot Apex under the Microscope.

Under the low power of the microscope it is now a simple matter to recognise the actual apex of the shoot, surrounded by some of the young leaves (Fig. 52). As the leaves of privet are arranged in alternating pairs, the apex, which is usually slightly dome-shaped, is seen to lie between the two youngest leaves which were borne on the axis in the plane of the section. At right angles to these would be borne the leaves of the second pair, but naturally the insertions of these do not lie in the plane of the section, though isolated pieces of this second pair are usually seen above the apex (Fig. 53). The leaves of the third pair are seen to be very large in comparison with the younger ones and to lie in the same plane as pair 1. Below the third pair there is a long region of axis before another pair in the same plane is encountered, and this corresponds with the region of the first two extending internodes. It is clear that the whole structure is of cellular construction, though the cells are small at the actual growing apex. Another striking point is that the youngest leaves and the apical part of the axis are the parts which appear most deeply stained. It will be remembered that in the dissection of the Brussels sprout bud the structures became more translucent



FIG. 52.—Photograph of a median longitudinal section of the shoot apex of privet
($\times 110$).

in appearance and more soft and fragile in consistency the nearer one approached to the actual apex, so that this appearance of density must be due to the treatment the buds have received since they were removed from the living plant.

Safranin and light green are suitable stains for these preparations. Safranin colours nuclei and woody tissues, whilst

the light green is a general stain and colours the cellulose walls and cytoplasm. Under the high power the apparently dense region is seen to be built of cells which have very thin walls, so that the dense appearance is due to the staining of the contents of the cells (Fig. 54*a*). These cells are thus shown to be filled with protoplasm, and in this is the conspicuous, red-stained nucleus, which may be recognised, with extremely few exceptions, in every cell of this region. The larger cells farther back appear less dense as many of them are quite unstained over a large proportion of their area (Figs. 52, 54*b*, *c*). Presumably large sap vacuoles nearly filled these cells so that the protoplasm was confined to a layer lining the wall of each cell. In thin

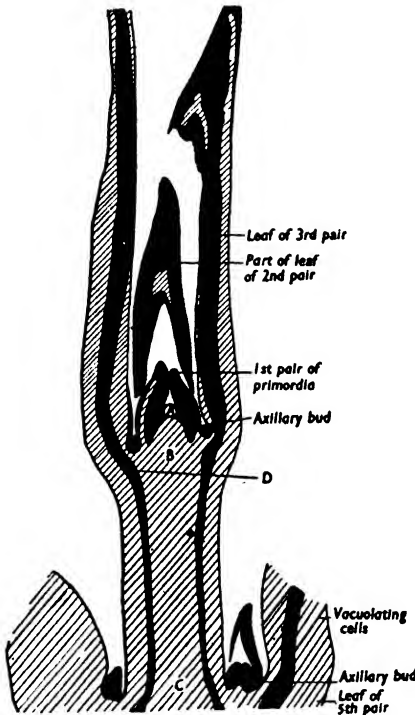


FIG. 53.—Diagram of the distribution of eu-meristematic and vacuolating tissue in the median longitudinal section of the shoot apex of privet ($\times 25$). Cells from A, B, C and D shown in Fig. 54.

sections (about 8μ or 0.008 mm.) these large cells may be cut through in such a way that they seem empty, but if a cell is cut so as to be seen in surface view in the section, the lining of protoplasm appears to fill the cell. In this older tissue also a nucleus is not seen in every cell. In such vacuolated cells, however, the nucleus lies, as in the snowberry cells, in the cytoplasm and often near the wall, so that when such cells are cut, as many of them are in these thin sections, the part

containing the nucleus is often missing, though maceration methods would show that each cell had a nucleus. We thus reach the very important conclusion that the cells of the most apical and youngest region are small and without prominent vacuoles, whilst the majority of the cells farther

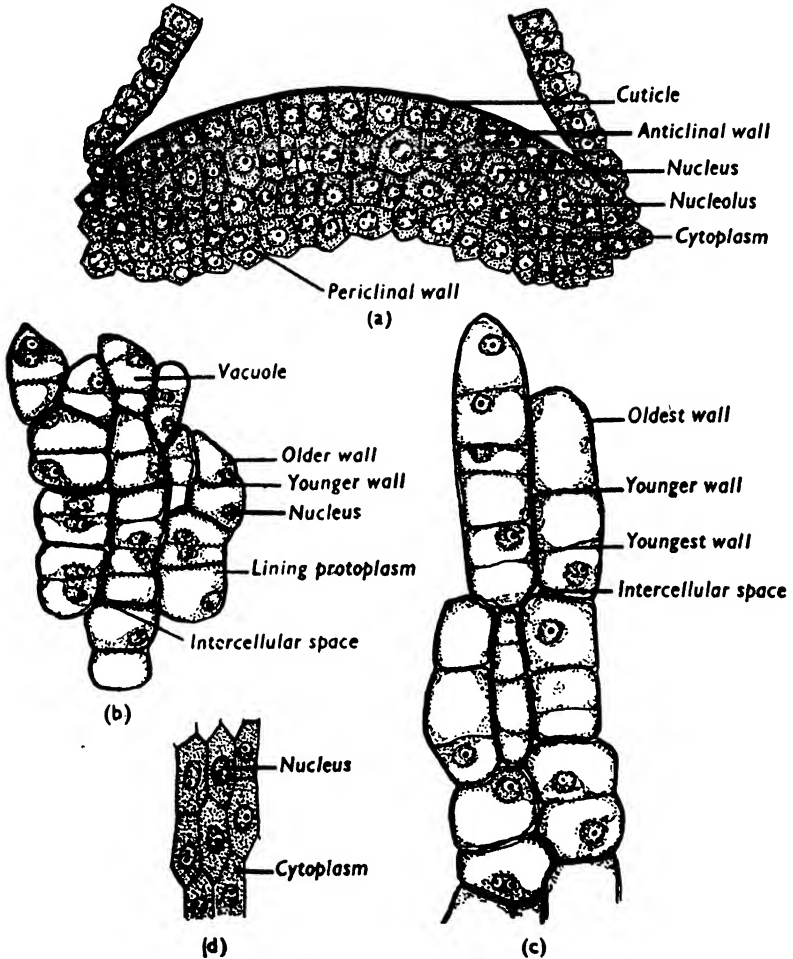


FIG. 54.—Types of cell composing the shoot apex of privet ($\times 580$). (a) Eu-meristem cells of the apex (from A in Fig. 53), (b) vacuolating cells from B, (c) vacuolating cells from C, (d) procambial cells from D.

back are larger and vacuolated. Behind the region of dense cells there is usually a relatively sharp transition to the vacuolating type of cell. Growth is proceeding by cell division and cell enlargement in both these regions of the bud and the tissues

composing them are described as meristem, a term which signifies dividing into parts and therefore refers to one of the most important features of the cells. Since the properties and manner of growth of these two regions of meristem show significant differences, they will be considered in turn and the region of dense cells will be referred to as eu-meristem to distinguish it from the region of vacuolating meristem. The eu-meristem is the first or initial type of meristem and it is from this that the vacuolating type is derived. The vacuolating meristem is meristem in the sense that the cells are actively growing and dividing, but other changes which these cells are undergoing may be regarded as stages in their differentiation into the permanent ground tissues of the plant.

The Eu-meristem.

The actual apex and the youngest pair of leaves are constructed of these small dense cells, which are most conveniently studied at the actual apex where they are seen to be present to a depth of about four cells in a median section (Fig. 52). In addition to the thinness of the walls and the central position of the nucleus, which must be actually spherical as it appears circular in every cell, it is characteristic of this tissue that the cytoplasm filling the rest of the cell has no visible structural features to be recognised by usual fixing and staining methods.¹ On the surface of the dome, where the cells are in contact with the air, a very thin membrane may be seen outside the cell walls. This appears more refractive as it is a sheet of fatty nature, and is found to be continuous over the whole shoot. The growing cells of the shoot apex always contain oil; some of this is released from the protoplast and follows the normal habit of such fatty substances and accumulates at a water-air surface, just as cream collects at the surface of milk. Vegetable oil in thin films always dries to a varnish, and this thin layer on the surface of the shoot behaves in this manner and forms the developing cuticle.

The size of the eu-meristem cells is seen to vary very little and yet they are found at the apex of the shoot where growth is most vigorous. If one compares the youngest leaves with those of the slightly older pair beneath them, it is obvious

¹ By special methods of technique various kinds of inclusion can be demonstrated in cells of eu-meristem.

that the great increase in size is very largely due to the much greater number of cells. The only possible conclusion is that growth in this region is taking place by repeated multiplication of the cells, which must divide as soon as they reach a certain limit of size. If a number of sections are carefully examined, some cells will be seen in the process of division; the stages are easily seen as the nucleus looks conspicuously different during division. (The details of nuclear division will not be considered until Chapter XXXVII.) From the small range in cell size observed in this meristem, every cell must divide when it reaches a certain maximum size, and the two daughter cells thus formed must differ little in size. The multiplication of these cells must largely constitute growth in a multicellular plant, for when a cell divides and the daughter cells increase to full size it must mean that double the amount of living protoplasm has come into being. The cells we are studying in this meristematic region are therefore most actively engaged in growth for it is here that the most vital part of the living plant, the living protoplasm itself, is being rapidly constructed.

The shape of the cells is seen in section to be angular and appears to be 4, 5, or 6-sided, but in order to realise what this means it is necessary to reconstruct their appearance in the solid. It will be noticed that the walls of adjoining cells are in contact at every point so that there are no intercellular spaces between the cells. Also the walls of the individual cells are so thin that it would be easy to assume that the walls separating adjacent protoplasts were single and common to both, but actually, by macerating the tissue, each protoplast may be shown to have its own wall. The cellulose walls are very thin in places, and probably at these points fine threads of protoplasm from the two cells are in continuity, or at least in contact; these protoplasmic contacts through the cell wall are spoken of as plasmodesma.

If cells from macerated tissue are compared with the sections some conception of the shape of the cells may be formed. The isolated cells appear as rather irregular, many-sided figures, the facets of which are mainly hexagonal or square. The sections show that the cells have angles of approximately 120° where they meet one another and that the cells occupy the space in the growing apex completely. All the cells are

growing and so they must all be pressing upon one another, and it appears that in this region the contents must be fluid enough and the walls sufficiently plastic for the cells to deform under this mutual pressure so that they occupy all the space. A simple experiment will illustrate the shape of these cells. A small cup (the metal cup of a thermos flask is very suitable) is filled with a large number of small plasticine balls of equal size, which have been well dusted with French chalk. The balls are then firmly pressed down into the cup by means of the top of a glass stopper which nearly fills the mouth of the cup. Under this pressure the originally spherical balls are deformed to occupy the whole of the space at the base of the cup. On shaking them

out the separate lumps of plasticine fall apart, and those from near the centre of the mass will have taken up shapes very similar to those of the meristem cells, and often appear as solid figures with about 12 sides (Fig. 55). The shape of the cells is thus explained as the natural result of the growth and division of plastic bodies under mutual pressure.

The spherical shape of the nucleus also deserves some consideration. Though these killed and stained preparations look dense in the region of the meri-



FIG. 55.—Experiment with plasticine pellets to illustrate the form of the cells of the eu-meristem (see text).

stem, it will be remembered that the same region in a living bud appeared very translucent and watery, and we now find that the shapes of the cells may be explained if the contents are liquid and thus readily deformed. If the protoplasm is liquid, the spherical nucleus can be most readily understood as a spherical drop in a liquid medium. It is rather more highly refractive than cytoplasm, and other evidence shows it to be

slightly denser, but evidently the difference is not great as the nucleus always occupies a central position in eu-meristem cells and shows no tendency to sink on to the basal wall. The nature of such small objects is not easy to determine with certainty so that indirect evidence of this kind may be very important.

If the growing apex were not a living, changing structure, we might now feel that we had obtained a clear mental picture of the eu-meristem region. We can form some conception of the distribution, size, and shape of the cells, the nature of their protoplasmic contents and the kind of thin cellulose wall, pierced by fine protoplasmic connections or plasmodesma, which surrounds each protoplast. The difficulty arises when we try to visualise how the cells behave as a living aggregate. We speak of an aggregate of similar cells as a tissue, and in this meristematic tissue we know that every cell is engaged in growing and making more protoplasm, both nucleus and cytoplasm, until it reaches such a mass that it divides into two new cells, which soon grow to be exactly like the original one. In fact the cells are so much alike in this region that it very soon becomes difficult to recognise which cells have recently been formed by division. However, a careful study of a longitudinal section (Fig. 54*a*) does throw some light upon the way in which the cells have been dividing. For example, the outermost cells definitely form a layer, as they divide repeatedly by walls at right angles to the surface (i.e. anticlinal; walls approximately parallel to the surface are spoken of as periclinal). It will be noticed that the anticlinal walls in the outermost layer never continue straight inwards as the anticlinal walls of the next layer within. Unless it is assumed that the cells can "slip" relatively to one another after formation, this is clear evidence that all new divisions in this outermost layer have been by anticlinal walls, so that new cells are added to this layer (in the dicotyledon) only in the plane parallel to the surface, and divisions in this layer never add new cells to the mass within. The implication of this statement is very extensive, for it means that the whole surface layer which covers the shoot has arisen by this type of division since the first inception of a meristem in the embryo plant. The surface layer of eu-meristem, which has this habit of dividing by anticlinal walls only, is called the dermatogen, a name which implies that it is the layer which gives rise to

the skin or epidermis of the shoot. The next layer or layers of cells beneath the dermatogen also divide almost entirely by anticlinal walls, so that in median longitudinal section the region of dense eu-meristem appears to be constructed of a small number of layers (commonly about four) which clothe a core in which the planes of cell division are more irregular and transition to vacuolating meristem is commencing. Schmidt described these two regions respectively as *tunica* and *corpus*.

A point which needs brief discussion is the manner in which the growing and dividing cells in the eu-meristem adjust their positions to one another. It is clear that when a cell has divided, the new cells represent two expanding centres in the place of one original one. Every such division means therefore a new adjustment of forces in the expanding mass and some adjustment of shape and position. Unfortunately

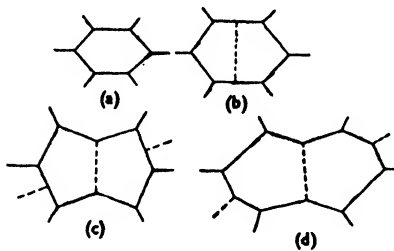


FIG. 56.—(a-d) Progressive changes in cell shape during the course of cell division in the eu-meristem as seen in optical section. New walls are dotted.

it is not possible to watch the process in a living plant, and the probable manner of adjustment can only be deduced from a study of the shapes of the cells of the eu-meristem at different stages. One suggestion is that the cells are able to move over one another, but the changes in position involved in such "sliding growth" do not seem likely when it is realised

that the cells have plastic cellulose walls and fluid contents and are lying against one another in a sticky layer of pectin. A more probable explanation is that the plastic walls would deform under the changing stresses until a new equilibrium position was attained, which appears to be when the walls meet at angles of 120° . A conception of the total changes involved in the tissue when one of the 14-sided cells divides is almost impossible to form, but the diagrams in Fig. 56 illustrate the type of change in one cell as seen in one plane. When a cell seen as 6-sided in section divides, the two new walls facing each other meet the wall of the parent cell approximately at right angles and two 5-sided cells result. By deformation of the walls, particularly at the point of junction, and divisions and readjustments of a similar kind in neighbouring

cells, it will be seen how 6-sided cells with the equilibrium position angles of 120° might again appear. Changes in shape and position must be proceeding continuously in the growing tissue, and the present interpretation assumes that it takes place without any slip of the cells over one another but by the "giving" of the whole framework of plastic walls, a type of adjustment which is described as symplastic.

On this view it is also clear that the outline of the plastic mass as a whole must be gradually and continuously adjusted in accordance with the rate of growth of the units constructing it. All the layers of the eu-meristem seem to grow at almost the same rate, a conclusion which is reached by determination of the percentage of cells found in any stage of division in the dermatogen or the deeper lying layers, in a series of sections. This being so, it will be realised that the surface layers, dividing entirely by anticlinal walls, will increase in area much more rapidly than the inner core of cells, dividing in all directions, will increase in cross-sectional area. The time must come when the surface layers will no longer be able to be accommodated as a smooth sheet over the summit of the dome-shaped apex. This results in a slow heaping-up of the surface layers, associated with a symplastic adjustment of the shapes of the cells following upon their division. The superficial fold thus produced in the plastic tissue will be a new leaf primordium. When the development of such a fold is studied under the microscope, the first sign of its appearance in the dicotyledon, is the elongation in a direction at right angles to the surface of one or two cells of the layer beneath the dermatogen at a point to the side of the apex. These elongated cells then divide by periclinal walls, and once divisions in this new direction are initiated other similar divisions follow so that a mass of tissue is raised on the flank of the apex. The dermatogen continues to form the surface layer over this upfold and to divide by anticlinal walls only. The processes leading to the formation of leaf primordia are evidently based on very orderly and characteristic sequences of cell growth and division in any particular growing apex, as we have seen that the leaf form and arrangement which ultimately result from this growth process are quite characteristic of the individual species.

The Vacuolating Meristem.

The cells behind the eu-meristem stain less deeply because a sap vacuole is present and the stained protoplast now forms a lining to the wall (Fig. 54*b*). Compared with the eu-meristem cells the vacuolating cells have obviously grown in size, but the increase in volume in this case is due more to the intake of water, less to the construction of more protoplasm. The nucleus now lies embedded in the peripheral layer of protoplasm and at first sight appears smaller than the nucleus of the eu-meristem cells, but closer observation suggests that this is mainly an impression produced by observing it in a larger cell. The shape of the nucleus also alters as, being a liquid drop, it becomes flattened against the wall in the thin layer of protoplasm. Whilst the protoplasm and nucleus are thus less conspicuous in the vacuolating cells, the walls on the contrary are thicker and more conspicuous. With increasing thickness the wall offers more resistance to expansion, and as a result the cells become rounded and separate from one another at the corners, leaving between them small intercellular spaces. In optical section these look like separate triangular spaces, but if the type of contacts made by a series of rounded bodies is examined it will be realised that these spaces are actually in complete intercommunication and form a continuous system through the whole tissue. This type of tissue occupies the central region of the shoot below the eu-meristem and also a more peripheral region, which is separated from the former by a narrow zone of cells which stain like the eu-meristem (Figs. 52 and 54*d*). At the surface the dermatogen maintains its eu-meristematic properties for a greater distance behind the apex, and thus intercellular spaces appear in the inner tissues sooner than they do in the surface sheet; this is also partly due to the fact that this layer has over its surface a thin sheet of cuticle and pectin, which dries and hardens in contact with air and thus renders the tearing apart of such cells more difficult. The absence of spaces from the outermost layer means that the spaces in the inner tissues do not immediately communicate with the outside air. These intercellular spaces appear between cells which are in a very early stage of vacuolation and which are full of water. Their walls at this stage are saturated with

water which freely penetrates cellulose, and the conclusion seems inevitable that these spaces when they first arise are filled with water. This water is subsequently displaced by air, as may readily be demonstrated by cutting a thin section of a living growing point under water or glycerine and examining it in the same medium. The air in the intercellular spaces will be seen as black lines or angular inclusions in the tissue, and these will be found to penetrate to a surprising height in the central pith and in the more peripheral cortical regions of the young shoot apex.

The walls of the vacuolating cells gradually grow thicker, but at the same time the cells also undergo divisions. The new walls around the daughter protoplasts are thin at first and in this way new walls may be distinguished. As a result it is possible to recognise groups of new cells, which have been formed by successive divisions of one original cell, and which are still held together for a considerable time within the stretched wall of the original parent cell (Fig. 54c). Such new cells will in turn round off as the wall reaches the stage when it resists the expansion of the contents within, just in the same way as the cells of *Pleurococcus* formed by division of an original spherical cell rounded themselves off in succession. In the present case, however, the cells do not separate completely, and, as nearly all the divisions are at right angles to the long axis of the shoot, long files of cells result and this phase of growth is consequently sometimes described as "file" or "rib" meristem (Fig. 54c). It is obvious that this type of growth and division in the vacuolating cells must be mainly responsible for the growth in length of the shoot.

In these two types of growth, that of the eu-meristem and of the vacuolating meristem, are found the fundamental features of cell organisation and growth which explains why, in the growing shoot, all the youngest leaves are crowded together at the centre of the bud at the shoot apex, whilst insertions of older leaves were separated by internodal extensions. The type of growth characteristic of the eu-meristem is responsible for the superficial growth at the shoot apex, which gives rise to the new leaf primordia and which, so long as they continue to grow in the same manner, remain crowded together. The growth of the vacuolating meristem is responsible instead for the growth in length of the shoot and the

extension of the internodes which separate the still growing primordia. The two types of growth are evidently fundamentally distinct though the change from one to the other occurs in the cells as they emerge from the eu-meristem, and close observation will show in any shoot apex a zone of transition from one type of cell to the other. In sections of fresh shoot apices mounted in iodine, the transitional zone may usually be recognised by the appearance of starch in the cytoplasm. The meristem forms mainly protoplasm at first, then for a brief period cells passing out of the eu-meristematic phase form starch as well and also more cellulose as shown by the thickening of the wall. The starch soon disappears, presumably being converted into sugar, which may be associated with the rapid entry of water to form more conspicuous vacuoles.

Evidently the growth of the eu-meristem cells is more strictly a growth process than the increase in volume of the vacuolating meristem cells. In the eu-meristem cell increase in volume means an increase in mass and mainly of protoplasm ; in the vacuolating cell, volume increase, though accompanied by an increase in protoplasm, is mainly due to the intake of water. Though it is customary to speak indifferently of any dividing tissues of the shoot apex as meristematic, in view of the fundamental character of the differences in cell behaviour just discussed, it has been felt desirable in this book to retain some distinction between true or eu-meristem and vacuolating or rib meristem.

The Fully Vacuolated Cells.

In the growing region all the cells may be described either as eu-meristem or as vacuolating meristem, but lower down the shoot, and later in the life of the cells, it is clear from the cessation of extension in the internode and of expansion in the leaf, that very many of the cells have ceased to grow. Whilst we have the characteristics of growing tissue in mind, it is worth while to consider whether this cessation of growth can be correlated with any new feature. Apparently green cells have the power of gaining in dry weight and of growth, so that when a cell of *Pleurococcus* or *Chlamydomonas* lay in a film of water exposed to light, there seemed to be no limit

to its growth and multiplication. Evidently the necessary materials for the synthesis of food reached the cells either from the air or through the water, in which were salts dissolved from the soil. So long, indeed, as a green plant lives in water, movements of solutes in the water seem to provide it with a constant supply of the raw materials necessary for life and growth. At the growing apex of the shoot the cells are not green, but they draw upon the food supplies stored in the cotyledons or made in the leaves. There remains, however, the question as to how these supplies reach the individual growing cells, a very difficult problem, incapable of a complete solution at present. There seems no doubt, however, upon one point, the food materials must move in solution in water and enter any living protoplast by diffusion from the watery fluid which bathes its cellulose wall. But these living, growing protoplasts of the shoot apex are raised far above the soil from whence the water supply must ultimately be derived. It is not clear how the walls of these growing cells continue to be bathed in a watery fluid or sap, but there is no doubt that this is the case. We may readily surmise that the "veins" of leaf and stem, the function of which we have to study later, have an active part to play in this respect. When the intercellular spaces first appear amongst the vacuolating cells, these spaces are full of sap and, as a result, sufficient food still reaches the vacuolating protoplast for growth and cell division to continue. Farther behind the growing point, however, the intercellular spaces are continuous with the system of spaces in the older tissues, in which the water has been displaced by air. Such displacement is only intermittent at first. Water still reaches the tissue and the protoplasts receive sufficient supplies of solutes to continue growth and division. Thus in the young internodes elongation is slow and mostly due to cell division. In older internodes a more rapid phase of elongation ensues (Chapter XIII), which is associated with a comparatively rapid increase in volume of the cells. Much water is then withdrawn from the intercellular spaces into the cells, whilst at the same time the expansion results in wider intercellular spaces. These spaces are now almost continuously filled with air, and the walls of the cells dry and thicken uniformly so that it is no longer possible to detect groups of cells in the files which

were originally formed from one cell. The supplies of water and solutes reaching the cells along their relatively dry walls are insufficient to permit of continued growth. Internode and leaf are now adult ; in the fully grown healthy leaf the protoplasts are still capable of synthesis and growth and are indeed actively engaged in making substances which are then available for the nourishment of the still growing regions. The adult cells themselves are not growing for lack of the full complement of supplies for growth, which could only reach them in water. In many cases, as for example after injury, when once more some of the intercellular spaces become injected with water, growth recommences in such cells when they are once more bathed in fluid.

Adult cells of this kind with cellulose walls and living contents form the main ground tissue of plants. Tissue composed of such cells, with which are always associated intercellular spaces containing air, is described as parenchyma ; in it the more specialised tissues, such as the veins, are embedded.

CHAPTER XVI.

THE STRUCTURE OF THE LEAF.

IN the longitudinal section of the shoot, the cells of the youngest pair of leaf primordia appear to be almost entirely eu-meristematic. In the second pair, of which only a part is seen in a median section in this plane, many of the cells are vacuolating (Figs. 52 and 53). The gradual onset of vacuolation in the successive pairs of leaves is, however, more easily followed in transverse sections (Fig. 57). Vacuolation is seen to appear first in the primordia of the second pair and in a position beneath the dermatogen on the outer or lower side of the future leaf. Very soon after this a second region of vacuolation appears within the dermatogen on the inner side, though this is much less extensive than that on the outer side. The two centres of vacuolation isolate between them a patch of meristematic tissue which eventually becomes the median vein of the leaf. In longitudinal view the vacuolating tissue of the leaf is seen to be dividing in the same manner as the tissues behind the eu-meristem in the axis of the shoot, though in the leaf the resulting files of cells are less regular, as divisions in other planes are more frequent. The result of the earlier vacuolation on the outer side is that the young leaves at this stage become arched over the apex.

The more prolonged meristematic growth on the upper side obviously means that more cells will be produced, and when these eventually reach the stage of vacuolation the curvature of the young leaves is reversed, and the leaves become expanded with the upper side approximately flat and exposed to the light.

When the expansion of the leaf has ceased, the adult lamina shows an intricate system of veins, between the anastomoses of which lie small "islets" of green tissue. Though parts of the

vein system will appear in any preparation of the leaf, these will be left for a later study, and attention will be confined at present to the tissues composing the islets.

Every leaf is a little different from any other in structure as in form, although in general features they may have much in common. It is desirable therefore to describe the structure with reference to the leaf of a particular species of plant, and

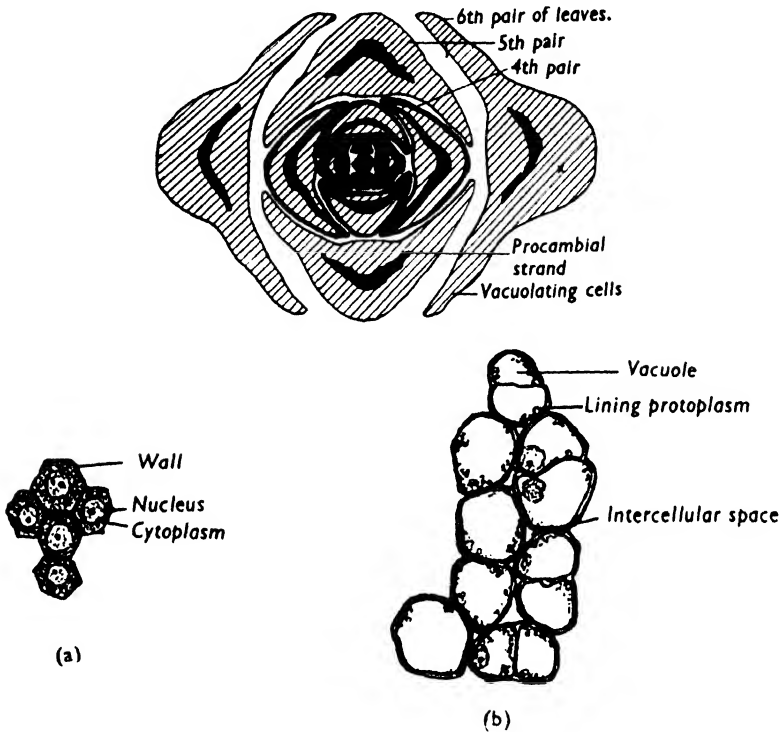


FIG. 57.—Diagram of the distribution of eu-meristematic and vacuolating tissue in the shoot apex of privet as seen in transverse section ($\times 40$). (a) Eu-meristematic cells from a leaf of the youngest pair. (b) Vacuolating cells from X ($\times 580$).

the present description will apply to the small evergreen leaves of box (*Buxus sempervirens* L.). Though other leaves may be employed, small evergreen leaves are particularly suitable for the maceration method.

In the first study of tissue structure it is very important that the solid structure of the object should be fully realised. By examination of the various tissues in more than one view,

drawings in two planes may be made; it is also instructive to model a small portion of the tissue in plasticine.

To obtain the necessary information the following methods of examination are suggested. A section of a fresh leaf is cut with a razor, and for this purpose the leaf may be held between the two surfaces of a longitudinally split piece of elder pith or carrot tissue. The sections are floated off into a watch-glass of water and then removed with the aid of a fine paint-brush and mounted in water or 10 per cent. glycerine. Under the microscope the tissues, with the exception of the outermost layer or epidermis on each side, look black owing to the air present in the system of intercellular spaces. This is an

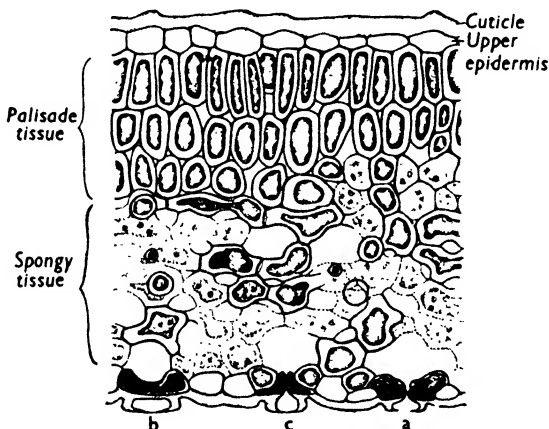


FIG. 58.—Transverse section of a leaf of box (the protoplasts shown contracted from the walls) ($\times 200$).

indication that such spaces in the living leaf already contained air, but the difficulty with which it is displaced by water suggests that the walls which abut on the intercellular spaces must be relatively dry. These walls are much more difficult to wet than the cellulose walls composing dry filter paper, so that the suspicion arises that they are greasy as well as dry. For the purpose of examination of the cells the air has to be removed, and this is done most effectively by soaking the section in 70 per cent. alcohol. The alcohol wets the walls more readily than water, which is also a further indication that the walls are greasy. The leaf of box has a closer texture than many leaves, so that a section needs to be thin for high-power

examination ; it is therefore helpful to have available some preparations cut from material preserved in alcohol, stained and mounted in balsam.

Even a very superficial glance at the section makes it clear that we have different types of tissue present in the leaf (Fig. 58). The two surfaces are bounded by regular cells, which seem to be extended parallel to the surface and which form a continuous layer. These are described as the upper and lower epidermis, whilst the intermediate tissue of living parenchymatous cells, amongst which also run the veins, is known as the mesophyll. The latter is also clearly much more regular towards the upper side of the leaf than towards the lower, and these two regions are distinguished as the palisade and spongy mesophyll respectively.

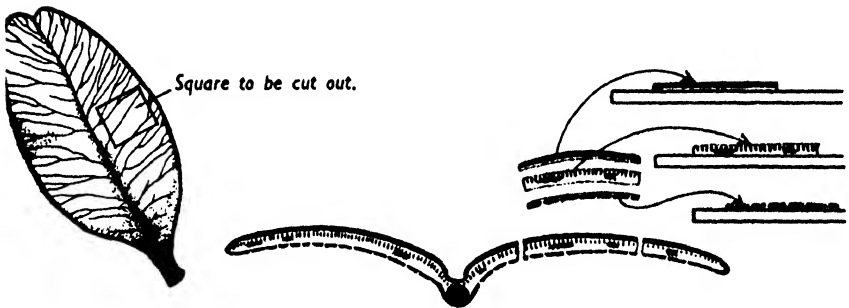


FIG. 59.—Method of mounting the three layers of a macerated box leaf (see text).

The more detailed description of the section is best considered in conjunction with views of the same tissues seen at right angles to the surface. For this purpose box leaves are taken which have been preserved in alcohol, as this removes the green pigment. Such leaves are carefully warmed in 8 per cent. potash ; they become fragile during this treatment and are spoilt if the liquid is allowed to boil. The warming is continued until the leaves begin to swell, an indication that the epidermis has separated from the mesophyll and a stage which is usually reached at the end of about 45 minutes. They are then washed gently in running water for 2 hours, and then soaked in 10 per cent. hydrochloric acid for several hours. After a final gentle rinsing in water the leaves may be stored in 5 per cent. formalin. The treatment breaks down the pectin cement between the cells of the mesophyll,

but if the time of warming is not too long, even these layers may be retained fairly intact if handled with care. The

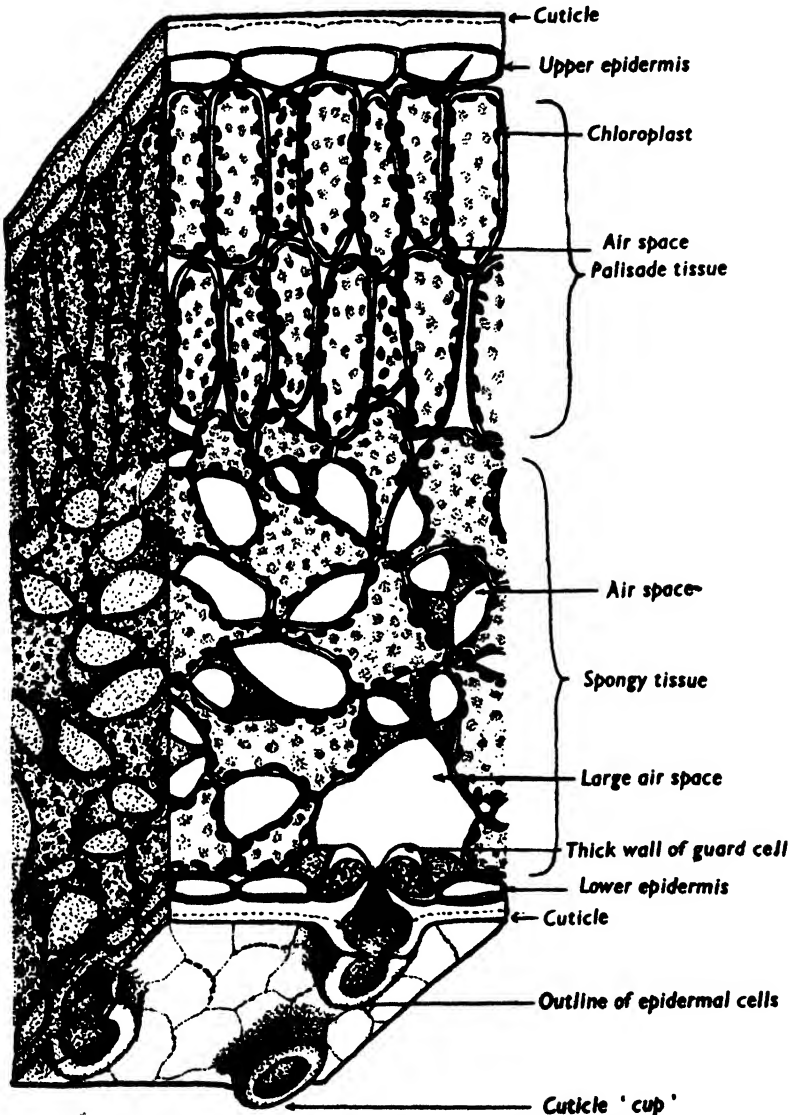


FIG. 60.—Structure of the box leaf reconstructed in the solid ($\times 400$).

potash disorganises the cell contents, some of which remains as an irregular lump in each cell, so that the preparations are mainly of use for the study of tissue construction.

After deciding which is the lower surface of the leaf, which is readily determined by the greater prominence of the mid-vein, a small square is cut from one side of the leaf, care being taken to have this free from leaf margin or mid-vein (Fig. 59). The piece is floated in a watch-glass of water, when it separates into three layers. These are carefully transferred with a paint-brush to a slide and mounted in 10 per cent. glycerine, the upper epidermis with the upper side uppermost, the lower epidermis with the outer or lower side uppermost.

Now that the section and the separated layers are available for examination, it is possible to consider each tissue in turn and to reconstruct its appearance in the solid (Fig. 60).

The Upper Epidermis.

In surface view (Fig. 61a) the cells form a very uniform sheet of polygonal cells, which are packed closely together without intercellular spaces. When the anticlinal walls are in optical section under high power, it can be seen that the thicker part of the walls is interrupted at frequent intervals by thin places, which give the impression of actual breaks. Cellulose is laid down only at a free surface of a protoplast, and the thin areas in the wall, known as pits, represent regions where the protoplasts of two adjacent cells have remained in such close connection (p. 201) that no free surface is present, and consequently the wall has not thickened. A section (Fig. 60) shows the cells to be about twice as wide in the periclinal direction as they are in the anticlinal. They are slightly rounded on the outer face and have one or two angles on the inner face, according to the way in which they press against the cells of the palisade mesophyll. The upper epidermis is thus constructed of a sheet of flattened cells, which fit together like a pavement of rather thick polygonal stones. The cells have been held together by the drying of a layer of pectins and fats at the surface, and consequently they have not been able to round off on vacuolation and no intercellular spaces have been formed. This fatty layer is visible in the section as a thick refractive cuticle overlying the epidermal cells and extending to a slight degree into their anticlinal walls. That the cuticle is mainly fatty in nature is readily confirmed by staining with Sudan III, prepared as a 0.1 per cent. solution in absolute alcohol diluted with

an equal volume of pure glycerine. This reagent stains oils directly, but in the case of solid fats such as the cuticle, the section is mounted in a drop of the stain and then warmed until the liquid begins to bubble. This is the temperature at which the alcohol boils, and it is usually about the temperature required to melt the fats in the cuticle sufficiently for them to dissolve the stain. On examination the fat soluble dye is seen to be confined to the cuticle and to have left the cellulose walls completely unstained. The nature of the cuticle is further demonstrated by placing another section in concentrated sulphuric acid. All the cellulose walls dissolve but the resistant layer of cuticle remains intact. Deprived of the support of the other tissues, this layer flattens out so that it is seen in surface view, when the pattern of the epidermal cells is visible upon it, as the fatty substances accumulate most where the anticlinal walls meet the surface.

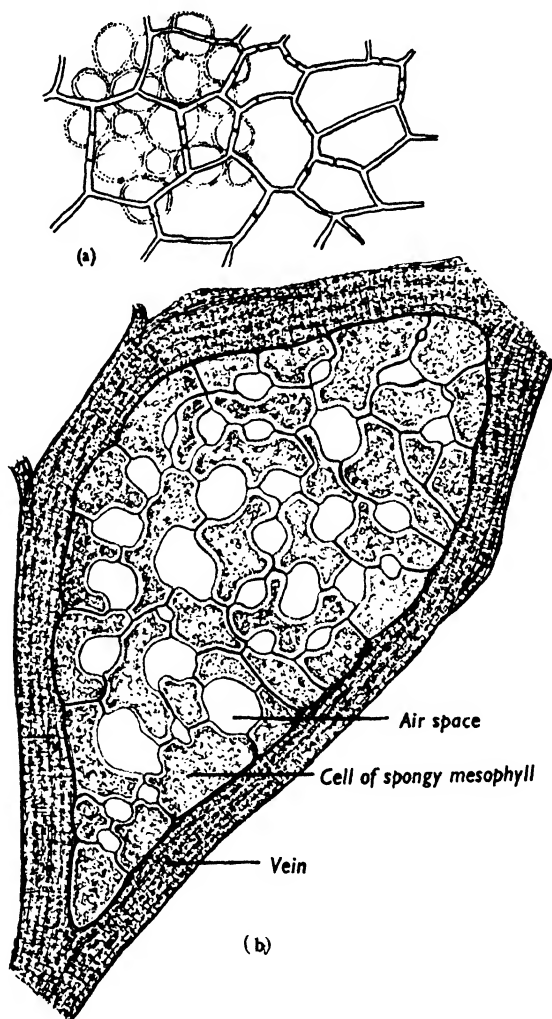


FIG. 61.—Box leaf. Tissues seen in surface view. (a) Upper epidermis, (b) spongy parenchyma, vein diagrammatic ($\times 180$).

The Palisade Mesophyll.

The section shows the palisade mesophyll as closely packed cells elongated in the anticlinal direction (Fig. 60). The two upper layers of this tissue consist of cells approximately twice as long as they are wide and which appear to fit closely together. Beneath them are one or two layers of squarish cells, which are more loosely packed. Although the cells in this tissue appear so closely packed in section, it must be remembered that sections of the fresh leaf showed air to be widely distributed throughout the mesophyll so that intercellular spaces must occur between them. In surface view the palisade cells are seen to be small and rounded, and if viewed through the upper epidermis (which is possible owing to the fact that some of the palisade tissue often adheres to the epidermis during maceration) their size is such that about four of them lie approximately beneath one epidermal cell (Fig. 61*a*). Small intercellular spaces are now very clearly seen between the cells, and these will run the full length of the cylindrical palisade cells and communicate with the air space system in the spongy mesophyll. In development the tissue just beneath the upper epidermis is the last to cease dividing, and consequently, when it reaches the stage of final expansion, the other tissues of the leaf have already undergone a good deal of their expansion, and the palisade cells under such circumstances naturally expand mainly in the direction at right angles to the surface of the leaf and the intercellular spaces between them are small.

In a section of a fresh leaf the green colour, which is deepest in the palisade, is seen to be due to numerous small green chloroplasts. These bodies occur in the peripheral cytoplasm of all living leaf cells, apart from the ordinary epidermal cells and some cells of the vein. In surface view each chloroplast is rounded, but in optical section it is seen to be flattened on the side towards the cell wall and convex towards the cell cavity. The closeness of the chloroplast to the cell wall has probably considerable functional significance. In Chapter XIX it will become clear that the process of gain in dry weight commences by the absorption of carbon dioxide in the chloroplasts. Their position so near the wall means that the carbon dioxide can diffuse in a gaseous medium almost to their surface. The

very short distance through which carbon dioxide has to diffuse, dissolved in water, means that the relative slowness of movement of this gas in a liquid medium does not impede the movement of the gas to the chloroplasts.

The Spongy Mesophyll.

The spongy mesophyll cells are very irregular in shape both in section and in surface view; they touch each other only at the ends of blunt arms which project from the cells in all directions, and therefore large intercellular spaces are present between them (Figs. 60 and 61*b*). In this tissue also lie the veins, the structure of which will be considered later. The mesophyll cells fit closely with one another over the surface of all veins so that, prevalent as intercellular spaces are in this tissue, none of them actually come in contact with the veins.

Lower Epidermis and Stomata.

Surface view (Fig. 62) and section (Fig. 60) show the lower epidermis to consist of cells similar to those of the upper epidermis, together with numerous pores or stomata. These pores are seen in section as small openings between two guard cells, which differ from other epidermal cells in that they contain chloroplasts. Around the pore the cuticle is raised into a curious cup. In surface view the pores appear as lenticular slits between the kidney-shaped guard cells but considerable differences in appearance are caused by slight alterations in focus. The highest focus (Fig. 62*a*) shows an oval rim, which corresponds with the lip of the cuticular cup seen in section in Fig. 60, and a less sharply defined wider circle which is the shoulder of the cup. A slightly lower focus shows the two kidney-shaped guard cells and the epidermal cells around them (Fig. 62*b*). The curious point is also observed at this level that, whilst pits are present between adjacent epidermal cells, none are visible between epidermal cells and guard cells. At a still lower focus (Fig. 62*c*) the epidermal cells and guard cells become less distinct, but refractive masses of boiled contents (usually about three at each side of the stomatal slit) come into focus. These are actually the contents of small mesophyll cells, which are

closely associated with the guard cells along each side of the pore (Fig. 62*d*). The walls of these cells are difficult to see by focusing through the epidermis, but the cells are seen clearly in a section, which passes across the centre of the slit (Fig. 58*a*). As seen in surface view the slits of the pores lie in various directions, and consequently in the section some

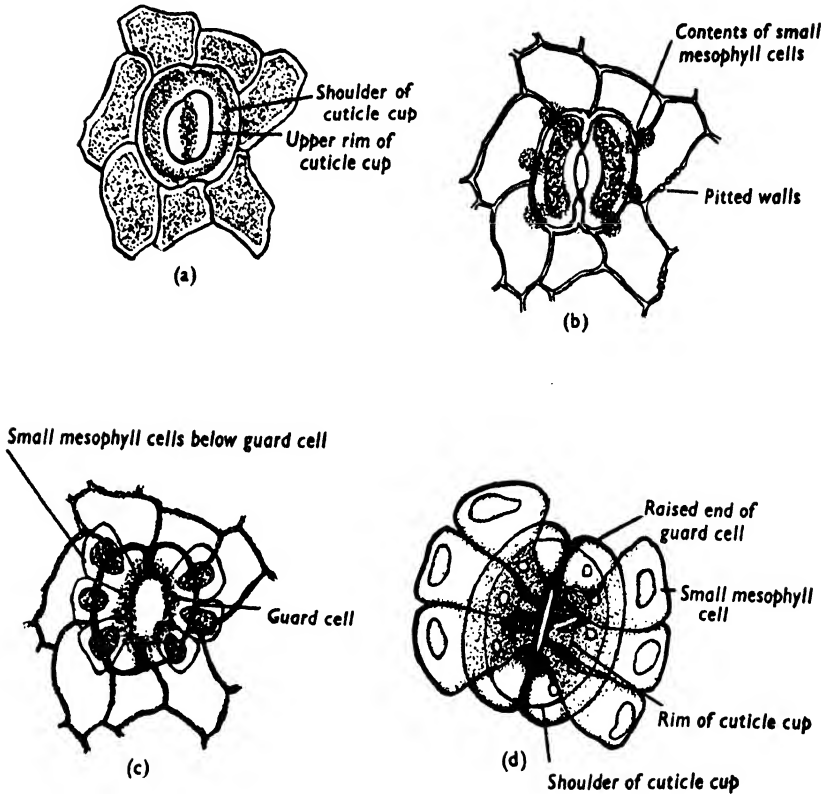


FIG. 62.—Box leaf. Successive appearances of the stoma on focusing downwards, highest focus at *a*, lowest at *d* (see text) (*a*, *b* and *c* $\times 400$, *d* $\times 700$).

stomata are cut at right angles to the pore and some parallel with it, whilst still others are cut in various oblique directions. In sections through the stoma in the plane of the pore a single guard cell is seen, concave on the side towards the leaf and somewhat enlarged at each end (Fig. 58*b*). The small mesophyll cells, lying below the stoma, abut on the guard cells

between their swollen ends (Fig. 62*d*). If the shapes of the guard cells are realised (Fig. 63), little difficulty will be experienced in understanding the different appearances seen on focusing on a stoma in a section cut at right angles to the pore. It is obvious that this may look very different according to whether the median part is focused, when the guard cells appear relatively flat and outside some of the small

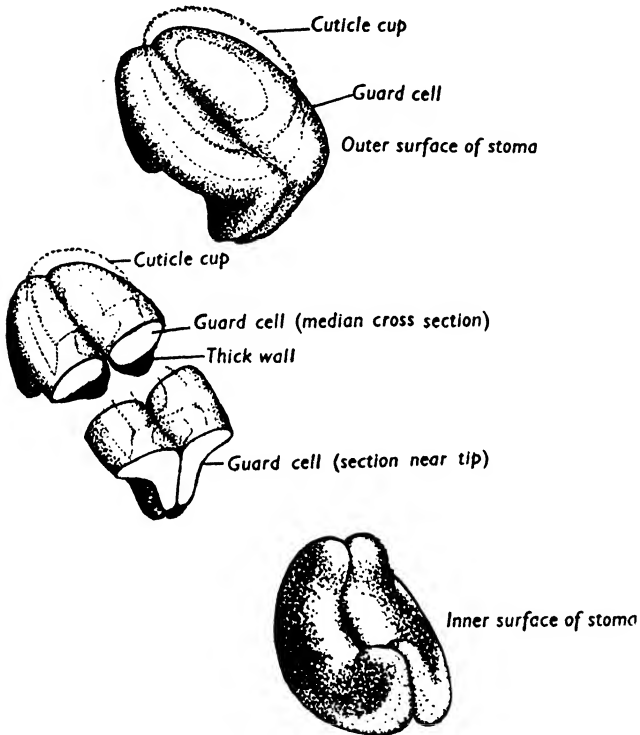


FIG. 63.—Reconstruction in the solid of the outline of the guard cells of the stoma of the box leaf.

mesophyll cells (Fig. 58*a*), or whether the end of a stoma is in focus, when the guard cells appear level with the small mesophyll cells (Fig. 58*c*).

The stomata are very numerous in the lower epidermis of the box leaf, but in the upper epidermis only occur over the main vein. Stomata are obviously of great importance in the normal functioning of the tissue, for through these apertures the internal system of intercellular spaces is put into

communication with the outside air. The possible significance of this is realised when one considers that the living protoplasts of the plant always function in a watery medium, and the true surface of the living system for most functional purposes is the surface where this water system meets air. Superficial observation might suggest that the important plant-air surface was the external form of the plant, but the microscope shows that the plant has a most extensive internal water-air surface. That such an internal system is in communication with the external air through the stomata may be demonstrated very simply. For this purpose leaves such as rose or hawthorn are very suitable as, like box, they have the stomata practically confined to the lower side. (This may be verified by observing the whole leaf under the microscope by reflected light, when the distribution of the stomata can be seen.) If such leaves are placed in hot water the air in the internal air spaces is expanded and is forced out as bubbles through the stomata. These bubbles appear very freely all over the lower side of the leaves, but are practically absent from the upper. It is therefore important to examine the stomatal pores in order to ascertain whether their area alters and if so in what manner they are controlled. This is not an easy matter to determine in the living leaf. If leaves are selected where the lower epidermis is free from hairs, by direct microscopic observation with reflected light (especially if use is made of the new objectives specially constructed for direct illumination of the object examined), it is possible to see that the stomata are usually open in the daytime but are closed in many plants if taken for observation at night. If the lower epidermis separates easily from the underlying tissues, strips removed and placed immediately into strong alcohol subsequently show a degree of opening of the pores which represents their state at the time of removal. Observations by this method confirm the direct observations by reflected light and are readily made over an extensive range of material.

Another method of demonstration of changes in the stomatal aperture is by means of a simple instrument known as the porometer. A small glass chamber with a flat-ground flange is fixed firmly to the surface of a suitable leaf by means of vaseline, gelatine, or other suitable adhesive. One tube

from this chamber dips under water in a small vessel, whilst another side tube, fitted with a tap, enables air to be drawn out of the apparatus so that water rises in the first tube. The latter preferably has a horizontal arm of narrow bore and is fitted with a scale. When the tap in the side tube is closed, the water level in the graduated arm slowly recedes as air enters the chamber above to restore the reduced air pressure to normal. The air can only enter the chamber from the intercellular spaces in the leaf through the stomata, so that the external air may be regarded as leaking inwards through this system of intercellular spaces, and the rate of leakage will be determined by the pore area on the leaf surface enclosed within the chamber of the porometer. In this way the plant with porometer attached may be put under different external conditions, and the readings of the rate of water movement in the tube should give a very good indication of the effect of these conditions upon the movements of the stomata.

The mechanism of the movement of the guard cells, which regulate the stomatal aperture, is probably a very complicated one, and a detailed analysis in every individual case would be necessary before it would be possible to estimate the influence of various factors. Both guard cells and epidermal cells may change in volume and the guard cells may also change in shape. In general, it is true that when water is withdrawn from the epidermal tissue the stomata tend to close, when water is freely supplied they tend to open. This can be demonstrated by stripping a little of the lower epidermis from a leaf of *Rumex Acetosella* L. and mounting one piece in water and one in 20 per cent. cane sugar. In water the stomata show a distinct opening bounded by a relatively broad, turgid guard cell on either side. In sugar solution the guard cells are relatively thinner and lie flat against one another so that there is no open pore between them. Of course it cannot be assumed that the epidermis in this experiment is behaving as in the intact leaf, but there seems little doubt that the stomata are normally open when their guard cells are fully turgid.

In this connection it is very significant that the guard cells, frequently alone of the epidermal cells, contain chloroplasts. These green granules often contain starch grains and,

as we shall see later, starch readily turns into sugar. Starch has little affinity for water and is usually in the cell in the form of an insoluble grain ; sugar, on the other hand, is very soluble and may be present in the cell sap and attract water into the vacuole. An increasing concentration of sugar in the guard cells thus means an augmenting tendency for them to take water from the neighbouring epidermal cells which are without chloroplasts and starch grains. When we add to these considerations the observation that starch is usually seen in the guard cells at night, or if the leaf is kept in the dark, whilst it disappears from them when the leaf is placed in the light, it seems clear that part of the complicated mechanism of stomatal opening and closure depends upon the carbohydrate supplies in the guard cells.

Our study of the leaf has shown us that the living plant has a most extensive intercellular air system, or system of air-water interfaces, and that the relations of this system to the outside air are controlled very largely by a complex mechanism of stomatal opening and closure. This works automatically under the impelling influence of a delicate balance of external and internal factors, which modify the water supply of the tissues and particularly the relative water supply of guard cells and neighbouring epidermal cells.

CHAPTER XVII.

METABOLISM. THE LEAF AS A CHEMICAL LABORATORY.

IN our study of the seedling we were led to the conclusion that the growing shoot used up material with loss in dry weight, but that as soon as adult leaves were present their activity was associated with gain in dry weight. When the material forming the dry weight of the plants was burnt, it gave rise mainly to carbon dioxide and water and left only a small proportion of ash, so that the bulk of it consisted of organic compounds, or in other words compounds of carbon. Now that the structure of the adult leaf has been examined we may consider more closely its connection with the process of gain in dry weight and therefore of formation of organic carbon compounds.

Our knowledge of these remarkable processes has grown very slowly, and we can still profitably consider a classical experiment, carried out in the seventeenth century, when chemistry was only just becoming a science and the elementary substances were still thought to be fire, earth, air, and water. Van Helmont (1577-1644) filled a large pot with 200 pounds of dried soil. In this he planted a willow branch which weighed 5 pounds. The surface of the soil was covered over to prevent the settling of dust, and over a period of five years nothing was added except rain water. At the end of this time the branch was found to weigh 164 pounds, whilst the dried soil weighed only 2 ounces less than at the beginning of the experiment. At this early date it was very naturally concluded that the gain was due to water. It may be assumed that the dry weight of the willow branch would have been roughly 50 per cent. of its fresh weight, so that over the period the dry weight increase was about 80 pounds. With our present knowledge

that this represents organic matter composed mainly of carbon, hydrogen and oxygen, it is probable that the hydrogen and oxygen were derived from the water, but the carbon cannot be accounted for in this way. In many of the substances which occur commonly in plants, such as sugars, starches, and cellulose, the carbon represents about $\frac{2}{3}$ of the weight, so that 80 pounds of dry weight probably includes about 30 pounds of carbon. Obviously this is far in excess of the loss in weight of the soil, which corresponds more closely with the increase in the ash constituents of the plant. This experiment shows conclusively that the carbon need not be supplied as complex carbon compounds in the soil, but is obtained from the carbon dioxide of the air. In 1840 Liebig in Germany gathered together the information available up to that time and stated that carbon dioxide, water and ammonia contain the elements necessary to build up the substances of plants. About fifteen years later Boussingault in France demonstrated that plants could be grown successfully in soil from which all the organic matter had been removed by burning, provided that nitrates were added to the soil water ; this left the air as the only possible source of carbon and also proved that normal plants are unable to utilise the free nitrogen of the air.

An experiment such as that of Van Helmont shows that the amount of carbon accumulated by the plant from the air may be very considerable, and at first this may seem very remarkable when one realises that the carbon dioxide in the air is only three or four parts in 10,000. There is, however, plenty of air and, owing to its construction, the leaf possesses a most extensive air-water surface. As soon as any carbon dioxide is removed from the air by a living cell, it is immediately replenished by the very rapid process of diffusion of a gas in a gaseous medium. There is no doubt that a green plant in the light can grow efficiently and increase in dry weight with no other source of carbon than the carbon dioxide of the air, a process which is sometimes spoken of as carbon assimilation, sometimes as photosynthesis.

It is not an easy matter to show experimentally that the carbon dioxide is absorbed by the green plant. The experiment was first carried out successfully by Boussingault and Dumas (1860-1890). Each day the air was analysed after it had passed over green plants in a glass vessel exposed to

the light, when it was found that green plants in the light removed carbon dioxide from the air. It has been related how Regnault, the famous physician, tested the accuracy of these early workers whose results, in dealing with such small quantities, seemed to demand precision beyond the powers then credited to the chemist. For some days the green plants had consistently withdrawn carbon dioxide from the air, but on a certain bright sunny day the two workers were astounded to find more carbon dioxide present in the air *after* it had passed over the green plants. Regnault then admitted that he was the cause of the trouble. In order to test whether they could really determine such small quantities of carbon dioxide, he had breathed into the apparatus whilst the experimenters had been out at luncheon !

This experiment has now been repeated many times and the results are always concordant ; in the light the green leaf absorbs carbon dioxide and gains in dry weight. The experiment is not, however, very simple to carry out as a class exercise. It is possible to illustrate the same point indirectly by using a green water plant such as *Elodea canadensis* Michx., when it may be shown that the plant in sunlight gives out oxygen, and that the process is dependent upon the presence of carbon dioxide in the water. Oxygen is not given off if the experiment is carried out with the plant in previously boiled water until carbon dioxide is once more added to it. If the experiment is carried out in fresh water during a period of bright sunlight in summer, and the gas from the plant led through a funnel into a test tube, this gas is often rich enough in oxygen to ignite a glowing splinter. In winter the gas collects more slowly and is not so rich in oxygen, as most of it is air which has been liberated from the intercellular spaces of the plant owing to changes in temperature. It may still be possible to show, however, by transferring the gas to a calibrated tube and then absorbing the oxygen in pyrogallic acid dissolved in potash, that the volume of oxygen in the gas collected is higher than is usual in dissolved air. (Dissolved air contains 34 per cent. of oxygen as compared with 21 per cent. in normal air, as oxygen is more soluble than nitrogen.)

The gain in dry weight of the green leaf, as the result of this absorption of carbon from carbon dioxide in the light,

may be demonstrated by a careful comparison of the dry weights of equal areas of leaf before and after some hours of exposure to light. The weights must be very exact, and mainly on this account, the technical difficulties in the way of securing reliable results usually make it advisable to leave this experiment to senior classes. By carefully controlled experiments, however, it is possible to establish with great precision the gain in dry weight of a green leaf in light in the presence of carbon dioxide.

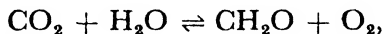
For instance, Sachs (1884) compared the dry weight of sunflower leaves gathered very early in the morning with that of leaves gathered at sunset. To make the results as comparable as possible, he took halves of the same leaf lamina on each occasion. In this way he obtained data from which he could calculate the gain in dry weight per unit area of leaf per hour. After making some allowance for other processes taking place in the plant at the same time, Sachs concluded that a sunflower plant with a leaf area of 1.5 square metres exposed to the light would increase in dry weight about 36 grams per day. This figure, in the light of later experiments, is probably rather high, but is of the same order of magnitude as is usually found.

If the dry weight of a plant is burnt and the carbon dioxide produced is measured, it is found that 1 gram of dry weight on burning gives 1.5 grams of carbon dioxide. The converse is roughly true also, so that 1.5 grams of carbon dioxide are required to supply the carbon to build up 1.0 gram of dry weight. It is of interest to consider what volume of air would be depleted of its carbon dioxide to supply the sunflower plant with carbon for the 36 grams of dry weight built up during one day. 10,000 litres of air contain about 3.5 litres of carbon dioxide (1 litre of carbon dioxide weighs 1.977 grams, approximately 2.0 grams). Therefore 10,000 litres of air contain approximately 7 grams of carbon dioxide. To make 36 grams of dry weight, carbon is required from about 50 grams of carbon dioxide. Therefore the carbon dioxide must be removed from $\frac{10000 \times 50}{7}$ litres of air = 70,000 litres approximately.

Actually the carbon dioxide is replaced by gaseous diffusion as rapidly as it is removed by the plant, so that no difference in concentration of carbon dioxide would be detected in the neighbourhood of the plant.

If green leaves are enclosed in a vessel containing a limited supply of air to which a known quantity of carbon dioxide has been added, it is possible on analysis of the residual gas mixture to determine the volume of oxygen released as the carbon dioxide is absorbed. In every case, within very close limits, the ratio of the volume of carbon dioxide absorbed to oxygen released is equal to 1. Therefore in the green leaf in the light, the gain in dry weight is connected with the absorption of carbon dioxide and with the release of one molecule of oxygen for each molecule of carbon dioxide absorbed.

As the green plant gains in dry weight, an analysis of comparable green plants, before and after such gain has taken place, might give an indication as to the nature of the substances built up in the plant from the carbon assimilated. Such analyses have frequently been made, comparing for instance green leaves picked in the morning with similar leaves picked at night, and the results point very definitely to an increase in carbohydrates as the result of this process of photosynthesis. Carbohydrates form a very large proportion of the dry weight of the normal green plant and it is perhaps not surprising to find that their formation is associated with carbon assimilation, particularly as many carbohydrates have a percentage formula CH_2O . This means that the formation of such a carbohydrate would lead to a gas exchange with the ratio observed :—



viz., one molecule of oxygen released for one molecule of carbon dioxide absorbed.

Though there is now very general agreement that carbohydrates are formed as the result of photosynthesis in the green leaf, the experimental difficulties in the way of a complete proof of this fact are very great and there is no certainty as yet as to which carbohydrates are actually the first to be formed in the process.

One carbohydrate that frequently appears in the leaf is starch, and for this there is a very simple test with iodine (iodine in potassium iodide solution) with which starch turns blue or blue-black. If a green leaf is killed by dipping in boiling water, decolorised in alcohol, and then treated with iodine, a blackening will indicate the presence of starch. Microscopic examination will show that all the starch is

present in the same granules which formerly held the green pigments, the chloroplasts. As it is only green parts of plants that are capable of photosynthesis, and as it will be found if part of the leaf is covered with tinfoil or black paper that starch is only found after a period of exposure to light in the unshaded parts of the leaf, the conclusion is very natural that starch is a product of photosynthesis. There can be little doubt of the general truth of this conclusion, but it is also true that starch can be formed in the dark from other carbohydrates, and consequently it is important to realise that observations, such as those just described, do not afford convincing proof that the starch found in the green leaf in sunlight has been formed directly from carbon dioxide absorbed from the air.

At present it is probably impossible to reach a definite conclusion as to the exact chemical changes involved in the process of photosynthesis. All these chemical processes which are associated with the continuous changes, both constructive and destructive, that lie at the very basis of life, are probably very complicated and take place in living cells, which, though very small, are themselves complex organisations. It is thus impossible to isolate for analysis a large bulk of any particular substance as it is formed in any one of the long series of linked changes that are in progress. As a result the chemist cannot satisfactorily isolate a substance, identify it and say with confidence that it is responsible for a particular stage in the changes observed. All that we can say at present is that certain classes of compounds play a very important rôle in the chemical changes associated with life, because they are always present in the living cells and the changes in the cells are associated with fluctuations in the amounts of these substances. Furthermore, we can see, as the chemical nature of the substances found becomes clearer to us, that they belong to classes or categories of chemical substances with certain general properties, which must be extremely important to them in the rôle they play in the living cell. Some of the substances of these classes may be isolated and then it is possible to study their general properties in the laboratory. Unfortunately a detailed study of their properties would require much time and considerable familiarity with the methods of physics and chemistry. Certainly the botanist is not going to understand the behaviour of the substances that

constitute the bulk of the dry matter of living organisms, nor their inter-relations with the water in which they must be dispersed in the living cell, unless he himself has some grasp of the viewpoint of these allied sciences and unless he is prepared to enlist with him in their study, other investigators who are more fully trained in these sciences. All that can be attempted now is to pass briefly in review three great classes of organic substances, carbohydrates, proteins and fats, which clearly play a large part in the processes of chemical change in the living organism. This attempt is made in order to see to what extent the conceptions of these substances, built up in the laboratories of chemistry and physics, help the biologist to form some sort of picture of the general nature of the changes which are proceeding in the living organism.

Metabolism is the general term used to cover chemical changes of any nature associated with life. It is a very useful term, as there are included under it many chemical changes which cannot be repeated or interpreted apart from the activity of the living organism.

When the nature of the great classes of compounds have been briefly analysed from the chemical standpoint, we can return to consider the few facts and observations at our disposal, which seem suggestive as to the course followed by the constructive metabolic changes associated with photosynthesis in the green leaf.

CHAPTER XVIII.

THE CARBON ATOM. CARBOHYDRATES, FATS, PROTEINS.

The Carbon Atom.

The characteristics of metabolic change depend very largely on the chemical properties of the carbon atom. As will be seen from the following survey, this element possesses very remarkable properties as regards its powers of chemical combination.

In the gas methane, one of the simplest of the compounds formed by carbon, we find the largest possible proportion of hydrogen combined with carbon. In this substance four atoms of hydrogen are combined with each atom of carbon, and we are probably justified if we picture the carbon atom as linked in four directions in space with hydrogen atoms. This conception can only be represented very inadequately

on paper by writing methane as
$$\begin{array}{c} \text{H} \\ | \\ \text{H}-\text{C}-\text{H} \\ | \\ \text{H} \end{array}$$
 The — joining

the carbon (C) atom with each hydrogen (H) atom represents a chemical attractive force, known as a valency or bond, which holds the two elements together. The strength of such chemical bonds varies according to the elements which they hold together; those elements with great chemical affinity for each other will hold together very firmly, those with less affinity less firmly, whilst there are some elements with no affinity for one another and these will not combine chemically at all.

In water, the chemist's analysis tells us that two atoms of hydrogen are combined with each atom of oxygen. It is therefore natural to find that a carbon atom, which may have

all its valencies satisfied by four hydrogen atoms in methane, may in other compounds have its valencies equally satisfied when combined with two oxygen atoms, so that $O=C=O$, CO_2 , or carbon dioxide, is a compound of carbon which is fully saturated with oxygen. These two elements have a very great affinity for one another, combine very readily and therefore release energy in the process, whilst on the other hand much energy is required in order to separate the carbon from the oxygen in CO_2 , since this involves the breaking of the chemical linkages holding these atoms together. In the following survey of carbon compounds which are of prime importance in living plants, it will be seen that in every case these four valencies of the carbon atoms must be taken into account, but since each valency may link the carbon with a different element or with a different group of elements, a very wide range of resulting substances is possible.

Carbohydrates.

Considering first the group of substances known as the carbohydrates, we find that the simplest substance which contains carbon linked with hydrogen and oxygen, in the proportions in which they occur in water, is formaldehyde, CH_2O . In this substance the four valencies of the carbon atom are

satisfied, $\begin{array}{l} H \\ \diagdown \\ C = O \\ \diagup \\ H \end{array}$, so that it can exist as a separate molecule

and as such it is a gas. In a compound of carbon in which one of the carbon linkages is with a hydrogen atom alone, this hydrogen is usually fairly readily replaced by other atoms or groups, so that the compound readily undergoes chemical change or, as we say, is reactive. In formaldehyde also there is only half the oxygen for which the carbon has affinity, so that this substance particularly easily undergoes changes which increase its oxygen content. Thus it readily changes

from $\begin{array}{l} H \\ \diagdown \\ C = O \\ \diagup \\ H \end{array}$ to $\begin{array}{l} H \\ \diagdown \\ C = O \\ \diagup \\ OH \end{array}$ or formic acid. In this process

formaldehyde itself is oxidised, whilst some other substance from which the necessary oxygen for the change has been taken is said to be "reduced." Towards other substances, therefore, formaldehyde shows very strong reducing properties and

the tests used to indicate its presence depend upon this fact. A commonly used test is to warm a little of the solution with an alkaline solution of a silver salt, when metallic silver is deposited as a mirror on the walls of the test tube.

If we write these two substances formaldehyde and formic acid, respectively, as $\text{H} \cdot \text{CHO}$ and $\text{H} \cdot \text{COOH}$ (always remembering that these are simply symbols to represent the way in which the atoms are linked together), then we can see that in each case we have a H-atom attached to carbon and thus replaceable by many other atoms or groups of atoms, in the first case combined with a characteristic grouping $-\text{CHO}$, the aldehyde grouping, and in the second with $-\text{COOH}$, the carboxyl or acid grouping. The properties of the acid group, their tendency to form salts with alkalis, solubility in water and sourness will be familiar to most, and in any case are better studied in the laboratory than through a long discussion on paper.

Besides the properties of reactivity and the power of acting as a reducing agent, another important property of formaldehyde is the tendency which the molecules show to link up with each other without the loss of any atoms; the atoms are simply rearranged in the process which is known as polymerisation. This is particularly important because in the process the H— in one molecule of $\text{H} \cdot \text{CHO}$ is replaced by

the $\begin{array}{c} | \\ -\text{C}- \\ | \end{array}$ of another similar molecule $\begin{array}{c} | \\ -\text{C} \cdot \text{CHO} \\ | \end{array}$. It is

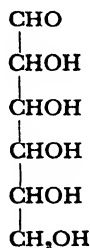
one of the peculiar properties of the carbon atoms that they have very great capacity for linking together and, once formed, the linkage between them is one of the strongest known. In the diamond and in graphite the substances are built up entirely of carbon atoms. In diamond the carbon atoms are linked with each other in all directions in space and they fit together so well that the arrangement has little tendency to alter and the substance is thus extremely stable. The diamond is consequently extremely hard, and when pressed against other substances such as glass, the atoms of the latter substances are more likely to shift their position than those of the linked carbon atoms, so that the diamond is the harder and will scratch the other substance. In graphite whilst the carbon atoms are again fully linked up with one another, the bonds

are so arranged that the linked atoms lie almost in one plane and there results a network of rings of carbon atoms. Each individual sheet of rings is very stable, but the sheets are only held together by the different and much less powerful forces which attract together the closely packed layers of atoms in a crystal. Since the individual sheets are so strong and are only weakly held together, the sheets readily slide over one another, so that graphite is an ideal lubricant, instead of a hard crystal like the diamond.

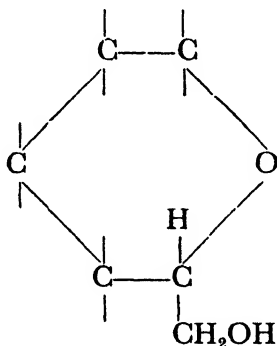
In formaldehyde the linking together of two molecules and the introduction of a $\begin{array}{c} | \\ -C-C- \\ | \end{array}$ linkage is very important,

because not only does the molecule become larger, but at the same time it gains in stability. Any number of molecules of formaldehyde might link up in this way, but owing to the way that the atoms are arranged in space, certain groupings of carbon atoms are more likely to arise than others, and doubtless that is why formaldehyde, in polymerisation, very frequently forms carbohydrates with six C atoms, which are spoken of as hexoses. Such a hexose may be written as follows :

where we see the original aldehyde group at the top of the molecule, with a chain of five carbon atoms in place of the original hydrogen atom of formaldehyde. It will be seen that the other five carbon atoms are represented as having attached to them a H—atom and an —OH or hydroxyl group. The organic chemist is able to characterise the hydroxyl group in certain ways ; thus the hydrogen in the —OH group may be replaced by the methyl group —CH₃ in the process known as methylation, and the possibility of adding so many methyl groups to this hexose molecule is in support of this general view of its structure. The hydroxyl groups have also great affinity for water and therefore a compound containing so many should be very soluble in water, a fact which is true of the hexose sugars. On the other hand, this six-carbon chain means a much larger molecule than in the gaseous formaldehyde, and it is in agreement with this fact that such a hexose, whilst readily soluble in water, crystallises out of solution as a stable solid.



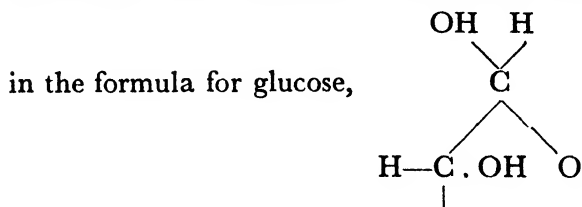
If the hexose sugar still retained on one of the carbon atoms the aldehyde grouping $\text{—C} \begin{array}{l} \text{H} \\ \diagup \\ \text{=} \\ \text{O} \end{array}$, it should still have strong reducing properties and be reactive. The hexoses are usually strongly reducing, in fact the reduction of the complex copper compound present in Fehling's solution on warming is the commonest test used to recognise such a sugar, but nevertheless they are more stable than the chemist thinks is compatible with this formula, and for many reasons the chemist now writes the carbon chain of the ordinary hexose, glucose, which occurs most commonly in plants as



in which the two additional valencies of four of the carbon atoms in the ring are satisfied with an H— and —OH linkage. When we write the formulæ for these substances in one plane on paper, we must remember that actually the carbon atoms are linked together in space; the angles at which they join, now known very accurately, render the five carbon atoms, thus linked into a ring through oxygen, a very stable arrangement which very naturally arises.

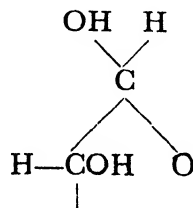
Upon the polymerisation of formaldehyde or upon the condensation of shorter chains of carbon atoms with the right proportions of hydrogen and hydroxyl groups, we might expect a particular compound such as the hexose sugar known as glucose to be formed. But one outstanding feature of this type of compound is its capacity to change, without the addition or loss of any atoms, into other allied but different forms, its isomers. This property again depends upon the arrangement of the various groupings attached to the carbon atoms

in space, because when a $\begin{array}{c} | \\ \text{---C---} \\ | \end{array}$ atom is linked to four different groupings, as for example, in the case of the top carbon atom

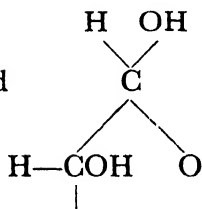


then the resulting compounds will be different if the hydrogen and hydroxyl groups occupy different relative positions in

space. Thus there are two different forms



and $\begin{array}{c} \text{H} \quad \text{OH} \\ \diagdown \quad \diagup \\ \text{C} \\ \diagup \quad \diagdown \\ \text{H---COH} \quad \text{O} \\ | \end{array}$ in which the relative positions of these



two groups attached to this asymmetric carbon atom may be regarded as mirror images of one another; they are known as stereo-isomers, and both always occur, because when one of these compounds appears, it tends to change into the other at a certain rate. This rate depends upon the temperature, so that, at any given temperature, a definite equilibrium mixture of the two compounds would be established in time. In the formula for the ring form of hexose, however, there are

five $\begin{array}{c} | \\ \text{---C---} \\ | \end{array}$ atoms which are attached to four different groupings

and can thus give rise to two stereo-isomers, and since each one doubles the number of different compounds possible, there is the possibility of 32 forms of hexose formed from one initial type in this way. Even this by no means exhausts the possible variety,

for other ring forms are known in which the linkage of the

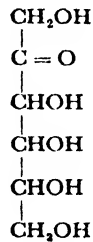
first $\begin{array}{c} | \\ -C- \\ | \end{array}$ atom through oxygen is with the fourth instead of

the fifth $\begin{array}{c} | \\ -C- \\ | \end{array}$ of the chain. Again other forms are known in

which the same atoms are rather differently arranged and the aldehyde group is replaced by a ketose arrangement :—

An example of such a ketose sugar is fructose.

It will be seen that it has the same percentage formula as the hexose types of glucose discussed above. Without going into the question in further detail, we see that when formaldehyde polymerises, or when by other processes carbohydrates with chains of from five to nine carbon atoms are formed, we pass from highly reactive, poisonous, gaseous or liquid substances to solid



substances, readily soluble in water, much more stable, though still somewhat reactive, and with the property of changing from one form into another very readily, so that the existence of one such substance implies the potential presence of a large number of forms, all with slightly different properties. The wide range of possibilities for metabolic changes concerned with such substances hardly requires stressing.

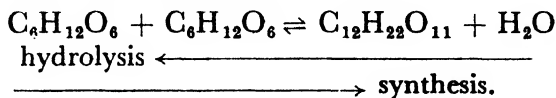
One change which readily takes place with carbohydrates such as the hexoses is of great importance. Two molecules of hexose, for example, will readily combine together with the elimination of water, giving a disaccharide,



This is not the formation of a 12-carbon chain from two 6-carbon chains, because the two 6-carbon chains are linked through oxygen. Sugars constructed of single carbon chains, which may range from bioses up to more complex chains than nonoses (9 carbon atoms) are all grouped as monosaccharides. In addition to the disaccharides with two carbon chains linked through oxygen, many more such carbon chains may be linked up through oxygen atoms, and will be discussed below. The difference is important for the new linkage

$\begin{array}{c} | \quad | \\ -C-O-C- \\ | \quad | \end{array}$, which joins the two chains or rings of 6 carbons,

is not nearly so strong as the $\begin{array}{c} | & | & | \\ -C & -C & -C- \\ | & | & | \end{array}$ linkage. In particular the linkage through $\begin{array}{c} | \\ -O- \\ | \end{array}$ is readily attacked by acids and the change is reversible. We shall speak of it as synthesis when it proceeds so as to build a larger molecule with the elimination of water, and as hydrolysis when the large molecule is broken down with the addition of water :—



Of the hexose sugars present in plants, glucose and fructose are familiar examples. Two molecules of glucose combine to give the disaccharide maltose, and conversely maltose is readily hydrolysed to glucose, whilst in the disaccharide cane sugar, one molecule of fructose is combined with one of glucose. Cane sugar occurs very commonly in plants and is readily hydrolysed into its constituent hexoses, but the way the cane sugar is constructed in the plant is still a problem, because the same synthesis cannot be produced in the test tube.

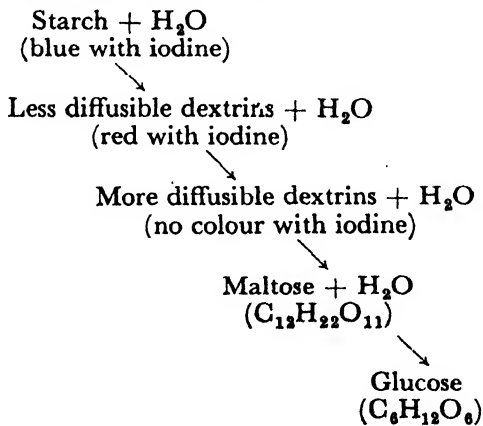
Such disaccharides as maltose and cane sugar are very much like the hexose sugars from which they are formed, but are larger molecules and therefore diffuse rather more slowly. It is evident, however, that such syntheses can be repeated again and again, each time with the elimination of water, so that from the original $\text{C}_6\text{H}_{12}\text{O}_6$ molecule, by the elimination of n molecules of water, we obtain a large molecule $n(\text{C}_6\text{H}_{10}\text{O}_5)$. We may write this process



and thus picture the processes that must be responsible for the construction of such substances as starch and cellulose, which have very big molecules of somewhat indeterminate and probably variable size but with this percentage formula. These macromolecules we may picture as long chains (commonly straight but branched in some components of natural starch); each link of the chain consists of a ring of five carbon atoms and one oxygen (as illustrated on page 236) and these rings are joined together by $-\text{C}-\text{O}-\text{C}-$ linkages. These long chains are also attracted to one another in the starch grain or in the cellulose wall by other forces which are similar to those which

hold the individual units of a crystal together, micellar or crystalline forces. The longer the chains the greater does the effect of these micellar forces become, and in cellulose and starch they are sufficiently strong to make it difficult to disperse the chains in water. Thus starch goes very slowly into colloidal solution and cellulose will not dissolve at all. Nevertheless many of the individual carbon atoms in the chains are still attached to hydroxyl groups with their strong affinity for water and, though starch and cellulose disperse slowly or not at all in water, yet water readily enters and swells the intact starch grain or the cellulose wall. These large complex molecules have certain new properties which arise simply from the size of the aggregate; they are very stable solids, which swell in water. Their stability renders them suitable to serve as forms of reserve food such as starch, or as constructional fabrics such as cellulose. Since they have been built up by repeated linkages of carbon groups through oxygen (syntheses) as in the disaccharide, they can therefore be broken down by hydrolysis into the original hexose sugars. Starch and cellulose can both be hydrolysed by acids to glucose.

If the hydrolysis of starch is carried out by some relatively slow method, it is possible to see that it progresses in a series of stages, more soluble and more readily diffusible substances being formed at each stage; amongst these are included the dextrans, some of which give a reddish colour with iodine, and then, finally, maltose and glucose.



When barley grains germinate, the starch grains in the barley are rapidly turned into maltose (glucose is not formed in any

appreciable quantity by barley), and if the germinating grains are minced up and extracted with water, an extract is obtained which, when filtered free from starch, has the power of bringing about hydrolysis when added to starch. This substance from the barley extract has never been obtained pure, but it is precipitated from the aqueous extract by alcohol. The dried and powdered precipitate, if redissolved still has the same property of bringing about the hydrolysis of starch. It is one of a large class of substances which apparently possess the power of facilitating a specific chemical change, in this case the hydrolysis of starch, without being permanently altered themselves in the process. They are known as enzymes or organic catalysts, and to the enzyme producing this change upon starch the name diastase was given many years ago. It is now realised, however, that in the germinating barley the "diastase," as with "diastases" derived from other sources, is composed of a number of enzymes, each of which is probably responsible for a definite step in the hydrolysis of starch as far as maltose. It is clear that this complex of enzymes called "diastase" differs from one plant to another in the relative amounts and potencies of the individual enzymes it contains. The system present in the fungus yeast also contains maltase, which completes the hydrolysis to glucose. Maltase is apparently absent, or very nearly so, from barley diastase. The hydrolysis or digestion of cellulose, when it occurs, is attributed to the presence of a hydrolysing enzyme cytase, of which too little is known to indicate whether it also is a complex of enzymes.

We have then some knowledge of the structure of such complex carbohydrates as starch and cellulose and of the methods by which they can be converted into simpler carbohydrates, because such complex substances may be extracted from the plant and their hydrolysis, either by acids or enzymes, can be carried out and studied in the test tube. However, no such substances as starch and cellulose have as yet ever been built up apart from the living plant, so that we are still very ignorant as to the method by which their synthesis takes place. Here, however, certain facts of observation and certain results of modern physical methods of study of such substances are worthy of brief mention. Starch is always found in the plant as a starch grain, whilst cellulose occurs

as a membrane. The starch is always formed within a living "protoplasmic" granule, the plastid (Fig. 64), which may be

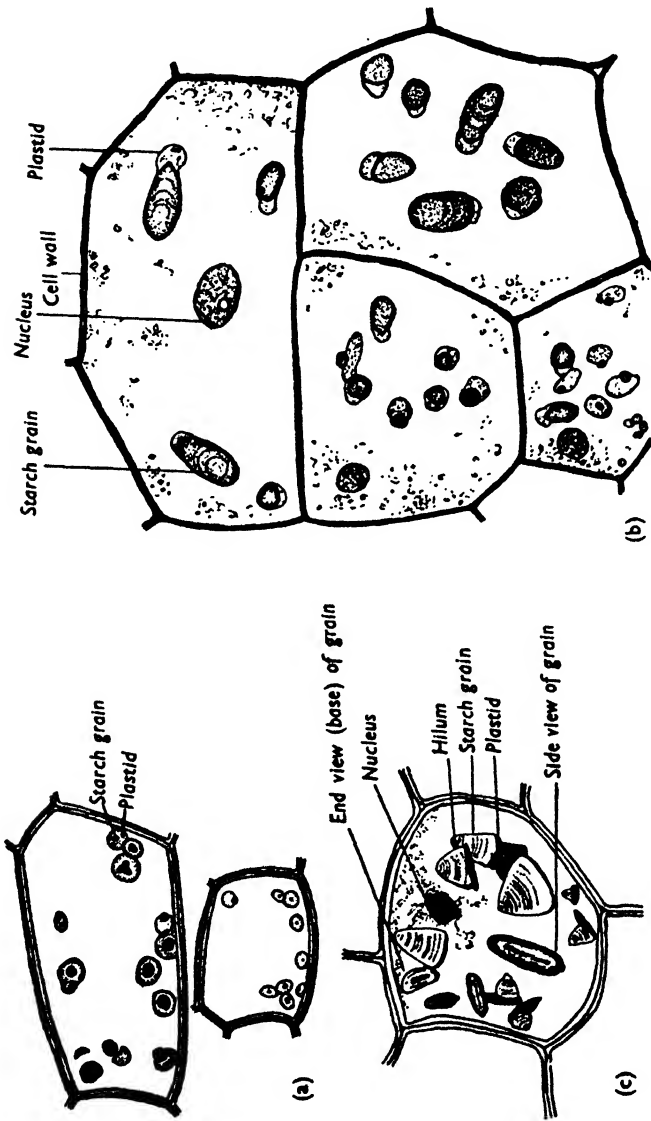


FIG. 64.—Starch grains within the plastid. (a) Early stages of starch formation in the chloroplasts of *Elodea*, stained in iodine ($\times 360$), (b) starch grains and plastids in a cortical cell of a young internode of *Pellitonia* ($\times 650$), (c) starch grains and plastids in a cell from the tuber of *Phajus* ($\times 150$).

green as in the chloroplast, or colourless as in the leucoplasts which occur in the potato tuber.

The green chloroplasts are very clear in the fresh leaf of the water plant *Elodea canadensis* Michx. In the broad cells

of the lamina they may be seen as circular green discs lying against the surface of the upper wall; on focusing down so as to obtain the cell in optical section, these discs are seen in side view closely pressed against the wall. If the narrow cells near the middle of the leaf are now examined it will be possible to obtain evidence that these chloroplasts are lying in the film of protoplasm that lines the walls of the cell, as in these long cells this layer is often in motion carrying the chloroplasts around with it. The movement of the chloroplasts along the length of the cell, around the end and back down the opposite wall, is striking evidence of the occurrence in these cells of the type of protoplasmic streaming known as rotation, as contrasted with the circulation of protoplasm between the surface layer and central protoplasmic mass surrounding the more centrally placed nucleus, which may have been observed when living cells of the snowberry fruit were under examination.

The early stages of the appearance of starch in the plastids may sometimes be recognised if young leaves of *Elodea canadensis*, which have been exposed to bright light, are decolorised in alcohol, treated with iodine and examined under the microscope (Fig. 64a). Starch first appears as a tiny spot in the plastid, but its growth soon makes it more conspicuous than the plastid, in which it usually appears to be excentrically placed. The young green internodes of the stem of *Pellionia Daveauana* N.E.Br. (Fig. 64b) and the fleshy green tubers of the orchid *Phajus grandifolius* Lour. (Fig. 64c) provide very beautiful stages in the development of the starch grain in which it is usually possible still to recognise the presence of the plastid. The fleshy leaves of the parasite *Lathraea Squamaria* L. (Chapter XXXI) also usually provide good material for recognising the starch grains still enclosed within a colourless leucoplast.

In the fully developed starch grain of a potato, the grain is a relatively large structure, filled with starch, around which the original plastid is reduced to a thin film, which, however, still completely encloses the starch (Fig. 65a). The plastid skin surrounding the starch is intact and prevents the starch from going into colloidal solution in the surrounding water. If potato tubers are grated into a fine pulp and the pulp is then kneaded in a linen bag in water, the grains will pass through the material into the water and leave behind a residue

of torn cellulose walls in the bag. The grains will settle out in the water and if the water is filtered off from the grains and tested with iodine, the absence of any blue coloration will show that no starch is present in it. But if the grains are now ground firmly in a pestle and mortar and then stirred up with water, this water, after filtering, will give a starch reaction as the starch has apparently diffused out through the damaged plastid skins of the crushed grains. It is not possible to demonstrate the plastid skins in grains full of starch, but if one is right in assuming their continued presence it would provide an explanation of the behaviour of starch grains on crushing and also on boiling. If some starch grains are boiled in a drop of water on a microscope slide they swell and some of the starch escapes from the grains as a colloidal solution which gives a blue colour with iodine, whilst the sac-like residues of the swollen grains stain a reddish-brown. In the past these sacs have been interpreted as the plastid skins through which the starch has diffused, but recently another interpretation has been put forward. We know that natural starch has two components; one, amylose, stains blue with iodine and its "molecules" are straight chains and the other, amylopectin, stains a reddish colour with iodine and its "molecules" are branched chains. It is suggested that the sacs represent the amylopectin components of all the lamellae in the grain expanded and pressed into a single layer.

Under the microscope the intact grains show a beautifully lamellated structure, with the layers arranged in roughly concentric zones around a dot, the hilum, which usually lies towards the narrower end of the somewhat oval grain. The hilum is evidently the place where starch first commenced to form in the plastid and since then around this point, layer after layer of starch has been added, with varying densities at different times, which accounts for the lamellated appearance (Fig. 65a). If pressure is exerted on the cover-glass, the grains are crushed and the cracks are always seen to radiate from the hilum, which suggests, therefore, that this spot is not a solid firm centre, but rather a small region empty of starch, so that the starch evidently originated as a layer around a tiny internal surface in the plastid. This surface then continued to become larger as more and more starch was deposited, but throughout the growth of the grain, we may

think of the starch as deposited continuously upon an internal, living surface. In an equally definite manner, the cellulose wall is formed by the living protoplast, and only thickens as long as the protoplast is alive, but in this case it is deposited upon the external surface of the protoplast. Both these complex carbohydrate structures are deposited upon living protoplasmic surfaces, which must presumably be continuously supplied with soluble carbohydrate (possibly hexose) molecules. The use of modern physical methods, namely the polarising microscope and X. rays, now tells us something more. If a starch grain is examined under the polarising microscope, the beautiful black maltese cross seen in the otherwise bright

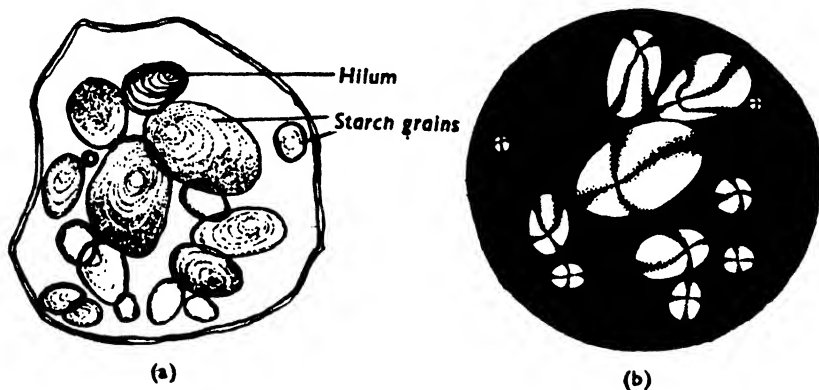


FIG. 65.—(a) Starch grains in a cell of the potato tuber ($\times 300$), (b) potato starch grains in the field of the polarising microscope with crossed nicols ($\times 300$).

grain (Fig. 65*b*) and centred around the hilum, enables the physicist to say that the molecules composing the grain are orientated in a regular manner in relation to the hilum, just as the molecules in a uni-axial crystal are arranged in relation to the axis of the crystal. In the grain the hilum is apparently an internal surface of the protoplast at which starch deposition began, and we can now say, about both cellulose and starch, that these substances are not only constructed by the continual linking together of carbohydrate molecules, but that these molecules occupy regular positions in relation to one another, so that they form a pattern almost as regular as that formed by the molecules in a crystal.

Such patterns are built up out of chains of linked carbon atoms in much the same way as the threads are arranged in regular manner in a cloth. A starch grain or a cellulose wall is then something more than a complex chemical compound, for in it not only are the atoms definitely orientated in space, but also the simpler carbohydrates, which are the units of construction, have been placed in their position in the whole in a definite order, which gives definite characteristics to the structure. Though they show certain features in common with crystals, neither starch nor cellulose can actually be regarded as such, because crystals have the power of "growing" by the orderly deposition of their substance out of solution, whilst neither starch nor cellulose can be constructed directly out of solutions of these substances. The various preparations of artificial silk and cellophane which are placed upon the market have been obtained by the deposition of cellulose from solution in various organic solvents, but these artificial preparations have not the same properties as those possessed by the natural cellulose wall. Neither starch nor cellulose can be formed out of solution by any process like crystallisation; the long chains of the complex carbon compounds are linked and arranged at a living protoplasmic surface, which seems to act both as the spinning machine, forming the thread, and then as the loom, which builds up the fabric, probably directly out of the simpler carbohydrate compounds, which are soluble and so reach the protoplasmic surface in solution.

Of recent years the physicist has been able, by the use of X-rays, to obtain a much more definite idea of the way in which chains of molecules are arranged in these structures, and as more light is thus thrown upon the intimate construction of the substances, we should understand their properties better, and possibly even the conditions necessary for their formation. This brief incursion into carbohydrate chemistry will have shown us at least how suitable these substances are to play a rôle in plant metabolism. From any one simple carbon linkage of the type first constructed, others naturally arise in practically unending series; more complex ones are formed from simpler ones by the elimination of water and these may give simpler ones again by the process of hydrolysis. This latter process we know can proceed at ordinary temperatures,

relatively rapidly and under the control of enzymes. The building up of the more complex substances in the living cell we understand far less, but if enzymes are present, apparently they are usually able to accelerate the process of synthesis as much as that of hydrolysis, according to the conditions existent in the system. In the case of the more complex carbohydrates, there is also evidence that their construction proceeds at special protoplasmic surfaces. The simpler forms are extremely reactive, so much so in fact that if formaldehyde is formed it must immediately change into something else, or otherwise it would immediately prove toxic on account of its strong tendency to link up with and alter some neighbouring part of the living chemical machinery. As the substances become more complex, they react less violently, but are still sufficiently reactive to be able to undergo changes, and are either very soluble or have great affinity for water. Thus the sugars, found all over the plant, are able to move in solution to the various centres where different metabolic activities are proceeding, and with their power of changing continuously into balanced mixtures of isomeric forms, provide the possibility of all sorts of different reactions, both constructive and destructive, which may form part of the ordered series of balanced changes to which we give the name life.

Carbohydrates, Fats and Proteins.

In any living plant cell we should find, if our methods of analysis were sufficiently delicate, evidence of the presence not only of carbohydrates, but also of substances belonging to two other great classes of organic compounds, the fats and the proteins. These are also built up of chains of carbon atoms, and unfortunately the recognition and identification of these different categories of organic compounds in such a complex mixture as occurs in a plant, demands a very high standard of technique and long experience in organic chemistry. As an example, the reduction of Fehling's solution, which has been given as one of the properties of the sugars with an aldehyde grouping, and which under standardised conditions can even be used as a measure of the amount of such a sugar present, cannot safely be used as a certain indication that sugar is present in a complex mixture, since other substances

with similar reducing properties may also be present. Reference to any suitable laboratory manual of organic chemistry will provide instructions as to tests characteristic of these different classes of substances, and here we will only discuss the simplest of tests which may be used as a basis on which to discuss the fact of the existence of compounds of these various groups in the plant.

Carbohydrates consist of relatively short carbon chains linked together through oxygen atoms, whilst proteins are similar carbon chains linked together through nitrogen atoms, and fats are simply long carbon chains, which can only be broken down by actually breaking the linkages between carbon atoms, and for this reason they are very stable. Some of the differences between the behaviour of carbohydrates and proteins will obviously be traced to the difference in the

behaviour of a $\begin{array}{c} | \\ -C-O-C- \\ | \end{array}$ linkage, as compared with a $\begin{array}{c} | & | & | \\ -C-N-C- \\ | & & | \end{array}$. We have seen that the $\begin{array}{c} | \\ -C-O-C- \\ | \end{array}$ linkage

is easily hydrolysed in the presence of acids, whilst it is very stable to alkalis. The $\begin{array}{c} | & | & | \\ -C-N-C- \\ | & & | \end{array}$ linkage on the

other hand, may be hydrolysed either by acids or alkalis, but particularly easily by alkalis. Use is often made of this fact in microscopic work when it is desired to study the pattern or structure of walls, which are carbohydrate structures. If sections of plant tissue are treated with Eau de Javelle, which is an alkaline oxidising agent, the proteins are broken down to simpler, soluble constituents so that the protoplasm disappears, whilst the carbohydrate walls resist the treatment and are left intact in the "cleared" section. All methods of preparation of paper pulp, which practically consists of pure residue of cellulose walls from plants, depend upon the same

relative properties of the $\begin{array}{c} | & | & | \\ -C-N-C- \\ | & & | \end{array}$ and $\begin{array}{c} | \\ -C-O-C- \\ | \end{array}$

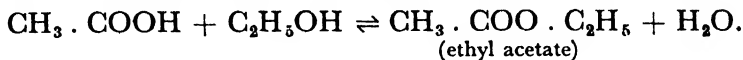
linkages. Another interesting difference between the two classes emerges when they are burnt. The carbohydrates, with no elements present except carbon, hydrogen, and

oxygen, are converted by the action of the heat into black, sweet-smelling, caramelised forms before they are burnt away completely to carbon dioxide and water. As a result, if sugar is burnt, the smell is like burnt toffee and comparatively pleasant. On the other hand, when proteins are burnt, the nitrogen present tends to link with hydrogen, forming ammonia or complex organic compounds which contain the —NH_2 (amino or amide) grouping, of which the smell is somewhat pungent, very characteristic, and definitely unpleasant. A typical smell of this type is that given by singed hair. If a broad bean or pea seed is burnt, this acid type of smell will easily be recognised, because in these seeds there is a relatively large quantity of protein reserves, whilst if a piece of apple or potato is burnt, the ammonia-like smell is scarcely detectable. Indeed, chemical analysis shows the remarkable fact that, though a cell can only live if it contains protoplasm, and though protoplasm has a dry weight of at least 70 per cent. protein, yet in such tissues as those of apple or potato, only a very small proportion of the dry weight is protein and the bulk is always carbohydrate.

Fats and Oils.

Since fats and oils have no linkages other than $\begin{array}{c} | & | \\ \text{—C—} & \text{—C—} \\ | & | \end{array}$ linkages in the main chain, they have few chemical characteristics and are usually recognised instead by their characteristic solubility properties. In the substances known as the fatty acids, the valencies of the carbon atoms composing the chain, which are not occupied with the $\begin{array}{c} | & | \\ \text{—C—} & \text{—C—} \\ | & | \end{array}$ linkages, are usually satisfied simply by being attached to hydrogen atoms, with the exception of the last carbon atom at one end of the chain, which is attached to an acid or carboxyl group —COOH . This carboxyl group readily reacts with the hydroxyl group of an alcohol, such as glycerol, and the resulting compound or ester is what is described as a fat. To illustrate this by taking the simplest case, we have acetic acid $\text{CH}_3 \cdot \text{COOH}$, which is a very simple acid of this type in which the H— of

formic acid, $H \cdot COOH$, has been replaced by $-CH_3$. This links up with ethyl alcohol C_2H_5OH to give ethyl acetate,



Although this is the typical reaction involved, yet ethyl acetate is not actually a fat nor acetic acid a fatty acid, because these substances have not the characteristic solubilities, which are features associated with the true fats or oils (the term fat is simply used to distinguish the members of this class which are solid at ordinary temperatures from the oils which are liquid; their chemical nature is similar). $CH_3 \cdot COOH$ is such a small molecule and the $-COOH$ group has such a strong affinity for water that it determines the solubility of the whole molecule, which consequently dissolves readily in water. The $-CH_3$ group itself has no affinity for water and as this part of the molecule is increased by replacement of one of these hydrogen atoms by carbons so as to give a chain of linked carbons $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot \dots$ then the true fatty acids are obtained, in which the $-COOH$ group at the end of the chain has great affinity for water, whilst the rest of the molecule has none at all. The result of this is that such substances have that property of collecting at water-air surfaces, spoken of as the "creaming" of fats and oils, when they form films at liquid surfaces. We now know definitely from X-ray studies that such films are arranged in such a manner that the $-COOH$ groups are in the water whilst the long "tails" of carbon chains project out into the air. This property goes far to explain why vegetable fats and oils, as they are released from the interior of the living cells, tend to appear at the nearest water-air surface and, in the shoot therefore, are found to accumulate at the surface. Another property is that they have the power of spreading and forming films over such surfaces; for example, they tend to spread over the cellulose threads in paper, so that one of the simplest ways to demonstrate their presence in an oily structure such as a seed like hemp (*Cannabis sativa* L.) is to crush the seed on to a piece of filter paper. A clear spot will be left on the paper, where the oil has covered the cellulose fabric and displaced the air. If the paper is held over a flame, it will be seen that the drop is not due to water as it only

tends to become more transparent and does not disappear. Other tests for fats also depend upon their solubility characteristics, so that the stains used to demonstrate the presence of fats are those soluble in fatty substances and not in water or in other substances with more affinity for water. Stains commonly used for this purpose are alcoholic solutions of chlorophyll or Sudan III. The latter reagent, in solution in alcohol, mixed with an equal volume of pure glycerine, has already been mentioned as a suitable stain for the cuticle on a leaf. In this case the sections had to be warmed until the dried fatty substances in the cuticle were partially melted before the stain would dissolve in them, but it is then firmly held, and the section, after being washed free of superfluous stain in water, gives a good demonstration of the red-stained fatty layer.

The cuticle is formed from the vegetable oils and fats, together with waxes (the esters of fatty acids with alcohols with a long carbon chain and a single —OH group), which have accumulated in a thin layer at the surface of the shoot. It is a well-known characteristic of vegetable oils that, if spread out in a thin film, as on a floor or on a picture, they tend to set to a varnish, which at first is "tacky" and later dry and even brittle. This property they owe to the tendency of the long carbon chains to link up together in the presence of oxygen into still larger molecules, by a series of condensations in which water is eliminated. This kind of linking up takes place especially readily when some of the carbon chains have linkages of the type that the chemist calls unsaturated, that is to say, that

in places $\begin{array}{c} | \\ -C- \\ | \end{array}$ is linked to $\begin{array}{c} | \\ -C- \\ | \end{array}$ by two bonds, instead of only one bond between the carbon atoms, thus $\begin{array}{cccc} & & H & & H & & \\ & & | & & | & & \\ & & -C- & -C= & C- & -C- & \\ & & | & | & | & | & \\ & & H & H & H & H & \end{array}$.

All vegetable oils contain a fairly large proportion of such unsaturated linkages and show, as a consequence, a strong tendency to dry to varnish-like products when spread out in thin films in the presence of air.

Proteins.

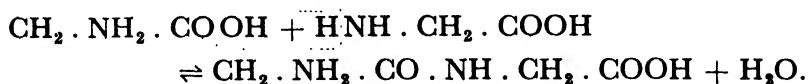
If plant metabolism were more fully understood, it would

certainly be necessary to give a much larger space, even in this introductory account, to the protein molecule. The building up of protoplasm is the basic phenomenon in growth and this involves the construction of protein molecules from still simpler carbon linkages. The simpler carbon groups, which are the units from which the proteins are built up, are the amino-acids, of which the simplest example is glycine or amino-acetic acid. As its name suggests it is acetic acid in which one hydrogen atom has been replaced by an amino group, $-\text{NH}_2$.

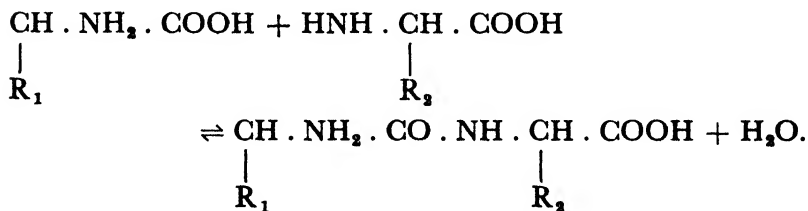
$\text{CH}_3 \cdot \text{COOH}$ is acetic acid and $\text{CH}_2 \cdot \text{NH}_2 \cdot \text{COOH}$ is glycine. Thus glycine possesses a $-\text{CH}_2-$ group, and in this either one or both of the hydrogen atoms may be replaced by other elements or groups. In the more complex amino-acids, one of these hydrogens is replaced by a carbon chain, which we may represent as $-\text{R}$, so that a general formula for an amino-acid is $\text{CH} \cdot \text{NH}_2 \cdot \text{COOH}$. It is character-



istic of an amino-acid that it always contains both a basic amino and an acid carboxyl grouping. As a result two molecules of amino-acetic acid may combine together by the interaction of these two groupings and, with the elimination of water, give a more complex molecule, glycine glycol,



Since all amino-acids similarly have amino and carboxyl groups, this is a perfectly general reaction, which will occur between any two amino-acids, and we may make the equation quite general by representing the carbon chains as $-\text{R}_1$, $-\text{R}_2$, etc.,



be extracted ; in the cereals some are present which have the rather unusual property that they can be extracted with 50 to 70 per cent. alcohol, in the pea and bean they are extracted best with water, and in hemp with 10 per cent. salt solution. An extract of hemp in salt solution is very suitable for the demonstration of one characteristic of such complex molecules. Though the protein in this extract is obtained in solution, the molecules are still large and will not diffuse through a parchment membrane or dialyser. If, therefore, a salt solution extract is placed in a dialyser, which stands in distilled water, the salt will diffuse through the parchment, so that on adding silver nitrate to the external solution a white precipitate is obtained. If the distilled water outside the dialyser is changed, the salt continues to diffuse out and the protein, which is insoluble in water, is gradually precipitated inside the dialyser. A little of this precipitate, mounted in iodine, shows that it consists of small protein aggregates, many of them crystalline in form, which stain yellow with iodine. Microscopic examination of seeds with protein reserves shows that the proteins are contained in special granules, known as aleurone grains. They are seen to advantage in the endosperm of *Ricinus* seeds. The simplest method is either to cut a thin section or to scrape off a little of the soft endosperm and then examine it in iodine. The preparation will look somewhat dense since the endosperm also contains quantities of oil, and these tiny droplets readily diffuse out into the surrounding aqueous iodine. Since the oil has a higher refractive index than the water, these droplets may be recognised by the fact that as one focuses up and down upon them, they become brighter as the focus is raised (cf. Chapter XIV). Around the edge of the preparation some grains will be sufficiently clear of the oil to be seen clearly. In such cases, within the oval outline of the grain, two associated bodies are present, a protein crystal stained yellow with iodine and a spherical body which is unstained and is in fact a mineral deposit known as the globoid (Fig. 66). Clearer preparations of the inclusions in the grain will be obtained if thin sections are first left soaking for a day in absolute alcohol, which removes the oil and enables them to be seen

much more clearly when the sections are then passed through water to the iodine solution (Fig. 66*b*). The protein crystal, of which there may be one or more in the grain, is called the crystalloid. Both the crystalloid and the oil around it have about the same refractive index as olive oil, so that if the material from the dry seed is mounted instead in olive oil,

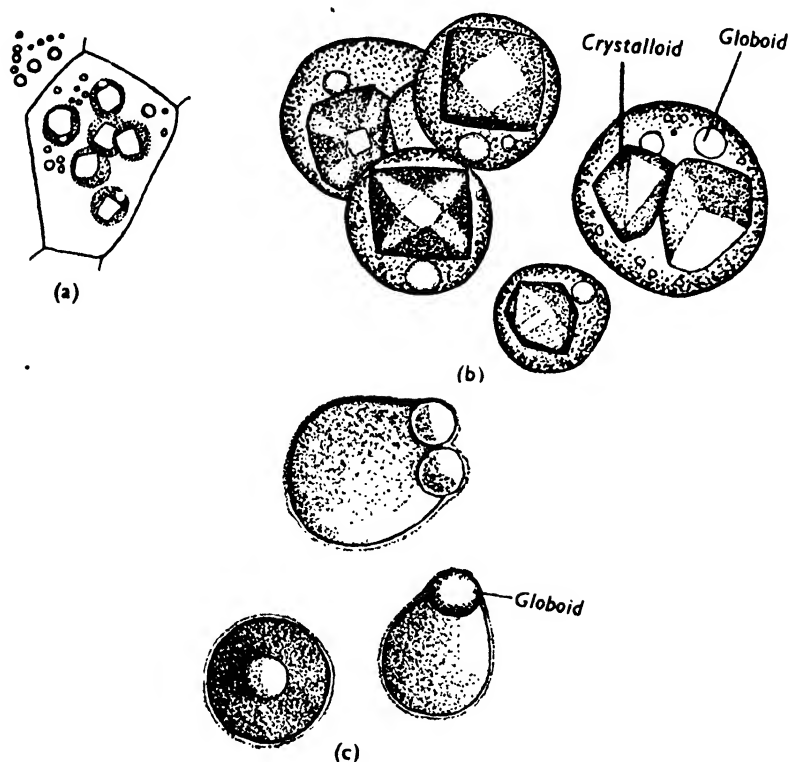


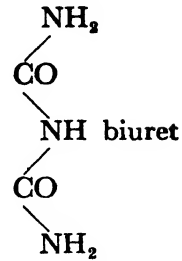
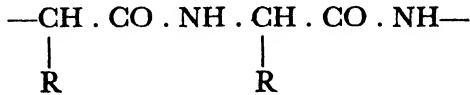
FIG. 66.—Aleurone grains from the endosperm of the seed of *Ricinus*. (a) Grains within the cell ($\times 400$), (b) grains mounted in iodine after previous extraction with chloroform ($\times 2000$), (c) grains mounted in olive oil ($\times 1500$).

the rounded globoid is the only inclusion visible (Fig. 66*c*); it has a lower refractive index than the oil and thus appears brighter at a low focus.

The whole series of laboratory tests which are suggested for the reserve proteins depend mainly upon the reactions of the constituent amino-acids. One important test, however,

depends instead upon the characteristic linkages of the main chain which joins together the various amino-acids. This biuret test depends upon a colour reaction with a copper compound, which is given by the biuret grouping : \longrightarrow

and is also given by any linkage of two —CO . NH— groups through either a carbon, nitrogen, or sulphur atom. It is therefore given by the proteins, where it has already been shown that the amino-acids are strung together by a linkage chain of this type,



To apply this test, strong caustic alkali is added to the protein extract and then a few drops of very dilute copper sulphate solution. In the absence of this characteristic biuret linkage, the copper simply forms a soluble, deep blue salt, but in its presence the solution turns instead a violet or reddish-purple colour.

Other reactions of proteins most commonly used by the botanist depend upon the presence of an amino-acid which has a benzene ring in it ; the amino-acid tyrosin, for instance, gives the reaction characteristic of such aromatic compounds. A reaction of this kind is the xanthoproteic test ; when strong nitric acid is added to the protein a yellow colour is obtained (the same colour is given by our own skin), and if this is followed by strong ammonia, a deep orange-brown colour appears. (The non-chemist should be very careful with this test as the reaction between the acid and strong ammonia is very violent. The nitric acid should all be poured away from the test tube before the ammonia is added, and further, if the test is carried out upon some solid substance, this may be washed with water, after treatment with nitric acid, before adding ammonia.) Another well-known test is to warm the substance with Millon's reagent (30 c.c. mercury dissolved in 570 c.c. concentrated nitric acid, then diluted with two volumes of water). The proteins precipitate with this reagent and turn red upon warming. Both these reactions can be illustrated very well by the addition of the reagents to fragments of bean or pea seeds.

For the purpose of these simple qualitative tests, fragments of plants which contain reserve proteins are best. If tissues of apple or potato are taken instead, the characteristic protein reactions are not readily given by the protoplasmic proteins present in the living cells : in fact the properties of the proteins which constitute the protoplasm, both nucleus and cytoplasm, are very little known, and are mainly deduced from the behaviour of the storage proteins and of their constituent amino-acids.

One amino-acid, cystine, introduces sulphur into the protein molecule, and this may be recognised by warming the protein with alkali in the presence of a soluble lead salt. The sulphur produces a blackening due to the formation of sulphide of lead. Phosphorus is not a constituent of any amino-acid but it is present in the molecule of nucleic acid, a complex organic substance which is always linked with the proteins of the nucleus. It seems certain that the remarkable properties of nucleus and cytoplasm are largely due to the properties of these complex proteins of which they are in large part constructed. This does not mean that protoplasm is a protein, but rather a wonderful, self-perpetuating equilibrium of substances (of which the bulk consists of labile forms of proteins), continually changing and yet ever maintaining its striking characteristics.

Here again, as with the carbohydrates, we see simple, soluble, reactive molecules, which link up into more stable structural complexes, which, though still changing in their relative proportions, etc., maintain a balance in which such metabolic changes can still continue. At first sight, perhaps, the structural features which build up the watery, transparent, highly refractive nucleus and cytoplasm would not appear to be such permanent structures as the cellulose wall or starch grains, or even as the aleurone grains of reserve proteins. A little reflection, however, shows that in these little understood, complex linkages of carbon chains through nitrogen atoms, etc., is welded something which has more unyielding permanence perhaps than iron or steel. In these complex carbon aggregates, in nucleus and cytoplasm, is handed on through the germ cells to successive generations the relatively unchanging characters of the species.

CHAPTER XIX.

PHOTOSYNTHESIS.

IN the last chapter the types of organic compounds which occur most commonly in plants have been discussed, and more especially in relation to those general properties of such compounds which probably account for the rôle they play in metabolism. We are now in a position to return to the original problem of the formation of these substances from carbon dioxide and water under the influence of light in the process of photosynthesis. When carbon dioxide is absorbed by the green plant an equal volume of oxygen is released, which would be the case if carbohydrates were formed, and sugars and starch are frequently found to be increased in the leaf after exposure to light. However, in view of what has been said as to the chemical behaviour of the carbohydrates, it will not be a matter of surprise that we are still in doubt as to which substance is first formed in the process, and as to the exact changes which it undergoes. We may, however, inquire rather more closely into the connection of the process with the green colour of the leaf and with exposure to light.

The pigments of the green leaf are not soluble in water and are not extracted even by boiling. On the other hand, if a red leaf, such as that of the red beet (*Beta vulgaris* L.), or a red variegated leaf of *Coleus*, is plunged into boiling water, the leaf turns green and the water becomes coloured by the red pigment which comes out into solution. Evidently this red pigment, an anthocyanin, though soluble in water, is retained in the living cells by the semipermeability of the living protoplast and is only released when the cells are killed (Chapter XIV).

If the green leaves, after killing in boiling water, are plunged into alcohol, the green pigments are extracted and the leaf

is left practically colourless. (The process is hastened if the vessel containing the alcohol stands in a bath of hot water.) This procedure is usually followed before testing a leaf for starch. If this is carried out with the thin leaves of the water plant *Elodea canadensis* Michx., subsequent microscopic examination shows that the plastids can still be recognised in the decolorised leaf (Fig. 64a); evidently the plastids did not consist simply of the pigments. If the fresh leaves are exposed to steam for a few minutes and then examined, the pigment will be seen collected in minute, oily droplets at the surface of the plastids; the latter seem to consist mainly of protein. The pigments, dissolved in an oily solvent, are held in the interstices of the plastids, much as an oil might be in a sponge.

As the result of a long series of researches, especially those of Willstätter and his colleagues, we now know that there are four different coloured components in the plastids, two of which are green pigments, chlorophyll *a* and chlorophyll *b*, and two yellow, carotin and xanthophyll. All the pigments may be extracted rapidly from air-dried leaves (finely ground, dry leaves of nettle, *Urtica dioica* L., are very suitable) with 80 or 90 per cent. acetone; the solvent is allowed to saturate the dry leaf powder for a time and is then drawn off by suction on a filter pump, and in this way a strong solution is readily obtained. It is a desirable precaution to mix the dry powder with powdered chalk before extraction, so that as the solvent extracts the pigments from the plastids, any acids in the tissues are neutralised before they come in contact with the pigments. This is very important, for a simple experiment will show how sensitive the pigments are to acid. The extracted pigments in acetone are first transferred to ether by adding ether, and then a small quantity of water, to the acetone solution. The liquid separates into an upper ether layer containing practically all the pigments, and a lower, almost colourless, acetone and water layer. In a separating funnel, the lower acetone layer may be run off and a drop of hydrochloric acid added to the ether solution. The green colour immediately changes to a dirty yellowish-brown, due to the loss of green colour by the two chlorophylls as they become converted into phæophytin. This change is now known to be brought about by the loss of magnesium from the chlorophyll molecule. Chlorophyll, whether *a* or *b*, is a complex organic

acid containing C, H, O, N and magnesium, combined with certain alcohols (methyl and phytol) as an ester. On treatment with acid the magnesium immediately leaves the molecule, and the dull coloured phæophytin is formed instead. The ether solution of phæophytin may be evaporated to dryness over a water bath, care being taken to avoid the presence of naked flames, and the residue redissolved in methyl alcohol. On warming with a trace of copper acetate the copper enters the molecule in the position of the original magnesium, and a new pigment is formed. This is bright green with a slightly bluer tinge than the original chlorophyll, from which it also differs in appearing green by transmitted or reflected light, whilst the fluorescence of chlorophyll makes it appear red by reflected light. This copper pigment is much more stable than the chlorophyll originally in the plant, a fact which is utilised in the preparation of museum specimens. The green plants are treated with vinegar (acetic acid), which removes the magnesium from the molecule, and then with a copper salt. The same effect explains also the common practice of adding sodium carbonate to the water in which green vegetables are to be boiled. When cabbage is boiled the green colour is often partly lost, because the sap of the tissue is sufficiently acid to decompose the chlorophyll, and this loss of colour may be prevented to some extent by the addition of the sodium carbonate before boiling.

The presence of the four pigments in the acetone solution may be demonstrated very simply by allowing the acetone solution to drip slowly from a burette on to a piece of fine-grained filter paper. As the drops spread out they carry the different pigments out to different distances in the paper ; the yellow pigments are carried out farthest, so that within the outer yellow rings are the limiting lines of the greens and still farther in, if present, the brown of the phæophytin.

Clearer separations of the pigments may, however, be effected by methods which depend mainly upon their different solubilities. To show that two green pigments are present, petrol ether is added to the acetone extract in a separating funnel, and then a small quantity of water. The liquids being immiscible, a separation into two layers takes place, with most of the pigments in the upper, petrol ether, layer. The lower layer of aqueous acetone contains only sufficient pigments to give it

a faint green tinge ; this may be run off. The remaining petrol ether layer is mixed with 90 per cent. methyl alcohol, when again separation into two layers occurs. Chlorophyll *a* remains in solution in the petrol ether, which forms a rather blue-green upper layer, whilst the chlorophyll *b* in the methyl alcohol appears a truer green colour. The exact colours of the two green pigments are, however, somewhat masked by the fact that the two yellow pigments which are present are also separated between the two solvents.

The yellow pigments may be separated by the following procedure. The pigments in acetone are first transferred to ether as previously described and then shaken for some time with a strong solution (30 per cent.) of potash in methyl alcohol. The shaking should be continued for about ten minutes and until the solution, which turns brown at first, again assumes a green colour. On addition of water, separation into a lower aqueous layer and an upper ether layer takes place. The usual effect of treatment with potash upon an ester is to break it down into its constituent acid and alcohols, the alcohols are released, whilst the acid forms a salt with the potassium. Such alkali salts of chlorophyll acid are soluble in water, and this incidentally explains why, when soda is added to the water in which cabbages are boiled, the water often develops a greenish tinge. In the present case these salts accumulate in the lower aqueous layer which also contains the potash and methyl alcohol, whilst the yellow pigments both remain in the ether layer. The lower potash layer may be run off and the ether extract washed with water, which is again run off from the separating funnel. The ether solution is then evaporated and the yellow, oily residue dissolved in petrol ether. On addition of 90 per cent. methyl alcohol, the rather more orange coloured carotin remains in the upper, petrol ether layer, whilst the xanthophyll passes into the methyl alcohol. These two pigments are both complex hydrocarbons, but xanthophyll, whilst containing the same number of carbon and hydrogen atoms as carotin, possesses in addition two atoms of oxygen. As these yellow pigments seem to absorb oxygen readily, this difference is suggestive, and especially so since chlorophyll *b* differs from chlorophyll *a* in containing one atom of oxygen more and two atoms of hydrogen less. As two atoms of hydrogen combine with

one atom of oxygen, this difference between the two green pigments is also equivalent to two atoms of oxygen. This certainly seems suggestive when we remember that these pigments take part in the complex photosynthetic process, in which a molecule of oxygen is ultimately released to correspond with each molecule of carbon dioxide absorbed, and also that these four pigments are always present together in the green chloroplast.

Although these pigments are always associated with the photosynthetic process, we have very little idea as to the exact manner of their functioning. It is obvious that the breaking down of carbon dioxide means the separation of two elements, carbon and oxygen, which have great affinity for one another, and consequently this cannot be accomplished without the absorption of energy. The energy presumably comes from the light, and the natural function to assign to the pigments is that of absorbing the light energy and using it for the work of separating the carbon and oxygen. This function also necessitates the presence of carbon dioxide in the chlorophyll system at the time, and here may lie the significance of the presence of magnesium in the chlorophyll molecule and of its great sensitivity to acids, even so weak an acid as carbonic acid. The probability is then that the light is absorbed by the chlorophyll and transformed into work in the separation of carbon and oxygen; the carbon is united with hydrogen and oxygen in the proportions of a carbohydrate, and once a simple member of this series is formed, we have seen from the general properties of substances of this group that it will immediately be transformed into other and more stable forms.

If light is passed through a prism the rays are bent in their passage, and in such a way that those of shortest wave-length are bent more than those of longer wave-length. Consequently by this means white light may be split up into the coloured rays which together constitute it, and the spectrum of these rays may be seen. If before passing through the prism the white light is passed through chlorophyll extract, it is seen that rays of certain wave-lengths are missing from the spectrum so that these have been absorbed by the chlorophyll. The absorbed rays prove to be chiefly a wide band in the blue and violet end of the spectrum and a band at the red end. If small screens

are made from Wratten and Wainwright colour filters, which allow only certain rays to pass, and these are fixed over a green leaf, it is possible to show after exposure to bright sunlight that starch is only formed under screens which allow red or blue rays to pass. This same point was illustrated in a much more spectacular way by a classical experiment carried out in Russia by Timiriaseff. By means of sunproof blinds, all light was excluded from a room except for one beam which was passed through a prism, after which the resulting rays were focused on a leaf. Using the same clockwork apparatus to compensate for the movement of the earth as that used by astronomers to keep a telescope focused steadily upon a particular star, it was possible to keep the rays focused steadily upon a particular part of the leaf. At the end of the day the leaf was decolorised and tested for starch, when the absorption spectrum of chlorophyll was found printed in the leaf in starch deposits of varying density. These experiments certainly suggest a close connection between starch formation in the green plastid and the light energy absorbed by the chlorophyll. It is important, however, to bear in mind

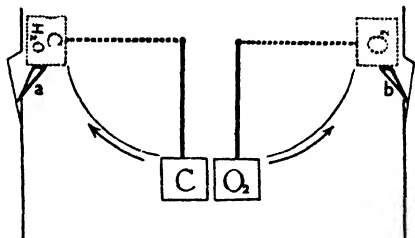


FIG. 67.—Diagrammatic analogy of energy relations in photosynthesis (see text).

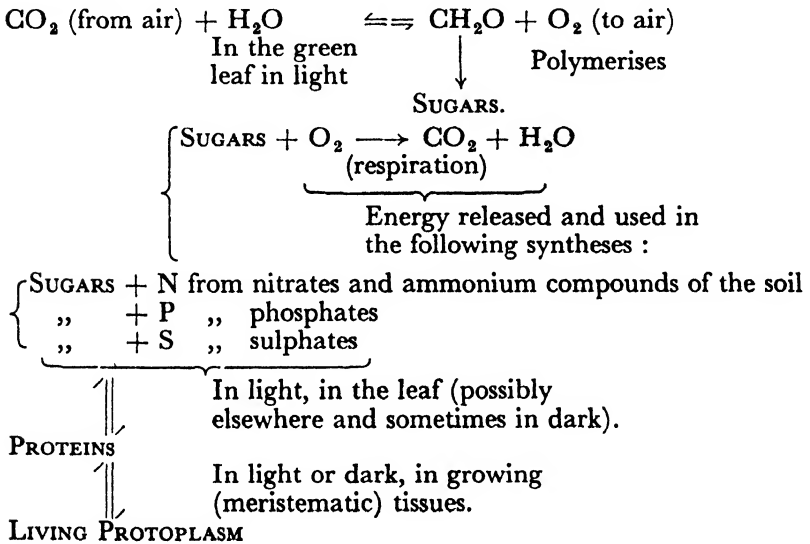
that the real connection between energy and photosynthesis is the necessity for energy to break down carbon dioxide. Provided that sugars are available, starch formation is able to take place in light or dark. Naturally, therefore, the necessary studies have been carried out to study the utilisation of carbon dioxide by the leaf when illuminated by light of different wavelengths, and such experiments support the conclusion that the energy absorbed by the green pigments is the energy used to break down carbon dioxide.

The energy used at this stage is also the energy *stored* in photosynthesis, because when once carbon dioxide is thus separated into carbon and molecular oxygen, the same amount of energy, as used in the separation, can once more be released by allowing the carbon to reunite with oxygen. Timiriaseff has put these facts in the form of a diagram, which

we may represent slightly modified in Fig. 67. Let carbon dioxide be represented by two heavy weights, which, at rest, lie side by side as in the figure. Force is required to separate them and the carbon and oxygen are so separated, suddenly and forcibly, when light falls upon carbon dioxide in the presence of the green pigments of the leaf. Like the weights in their new positions, they must be held apart or they would immediately recombine. The carbon, now probably in the form of highly reactive formaldehyde, and the newly released nascent oxygen would come together very vigorously to form carbon dioxide again. If we picture in our diagram two spring catches at *a* and *b*, which the supporting rods would pass easily on their upward swing, but which would not allow them to fall back, then without the continued action of the force, the weights would remain separated. These catches in the diagram are paralleled in photosynthesis by the changes immediately undergone by the carbon and oxygen. Highly reactive formaldehyde may polymerise to sugars, and from these starch may be built up, whilst the nascent oxygen, possibly passed from one pigment to another (pigments all differing from one another by just one molecule of oxygen), is ultimately released as more stable molecular oxygen. The result is that we now have stored in the photosynthetic products of the green plant the energy absorbed in the photosynthetic process. With the wide range of possible products involved in the process of metabolism, carbohydrates, proteins, fats, etc., in the presence of gaseous oxygen, to which they have a widely varying range of stability, there is represented the possibility of once more releasing this energy along a thousand different paths by once more allowing carbon and oxygen to unite. This reverse process will proceed relatively slowly, usually between substances in solution, and controlled in various ways by the complex construction of the living organism. The mechanism which controls the metabolic changes which subsequently release the energy is comprehended under the general term respiration.

The original separation of carbon and oxygen is the process which demands and stores the energy, and the natural assumption is that the carbohydrates first formed as a result of this process are the original stores of this energy and represent the accumulated gain in dry weight. But living matter is

more complex than mere carbohydrates and involves proteins, fats, and countless other compounds as well. Once organic compounds like sugars have been formed, from which energy can always be released in respiration, this energy is available for further constructive processes, and we are still quite uncertain to what extent the synthesis of proteins, etc., in the green plant is directly dependent upon the light and to what extent only indirectly through the utilisation of the energy already stored in sugars, etc. Although our knowledge as to these complex processes is still very meagre, we may tabulate the possible sequence of events in the following scheme :—



CHAPTER XX.

RESPIRATION.

OUR brief analysis of the metabolic processes of the plant may be completed by an examination of that other metabolic process, respiration, by which the organic compounds are again broken down to carbon dioxide and water whilst the plant loses in dry weight. This process may conveniently be examined after the discussion of photosynthesis, provided that it does not leave the impression that this process also is particularly to be associated with the adult leaf. Every living tissue is always respiring, and the more actively it is engaged in growth the greater in proportion is its respiratory activity ; growing points, for instance, respire actively and lose considerably in dry weight, whilst the adult leaf respire comparatively slowly and at the same time, in daylight, it is actively engaged in photosynthesis. Consequently the gas exchange and associated total metabolic changes proceeding in the adult, green leaf during the twenty-four hours have to be assessed as a kind of algebraic sum. All the time, comparatively slowly, the leaf absorbs oxygen and gives out carbon dioxide ; during the hours of daylight alone, it absorbs carbon dioxide and gives off oxygen and gains in dry weight as the result of photosynthesis. The photosynthetic process is so much the more vigorous that, although it persists over a briefer period, in sum the green leaf gains in dry weight and carbon is assimilated from atmospheric carbon dioxide.

Under the circumstances it is obvious that experiments upon respiration are carried out more simply upon tissues other than the adult leaf, and germinating seeds provide the most convenient material. If soaked peas are placed in a retort-shaped vessel (Fig. 68) with its mouth inverted over water, after two or three days the volume of gas in the vessel

appears to be practically unchanged. But if potash is introduced into the water and stirred to aid the solution of the carbon dioxide, the potash soon rises in the tube, showing that a large amount of the contained gas is now carbon dioxide. As the original amount of carbon dioxide in the air would be negligible, some other gas originally present must have been absorbed by the peas. The identity of this gas can readily be deduced from the height to which the potash may rise in the tube at the end of such an experiment. The volume of the tube occupied by the liquid will be found to be approximately one-fifth of the total volume of gas originally in the vessel. (This volume may readily be determined by filling the vessel, already containing the peas, with water and then pouring the water into a measuring cylinder.) As one-fifth of the normal air is oxygen, this is a clear indication that all the oxygen has been absorbed and as the level never rises appreciably higher, however long the experiment is left, the nitrogen evidently is not absorbed; it is also noticed that the peas soon cease to grow after the oxygen is exhausted. As the level of the liquid did not alter before the

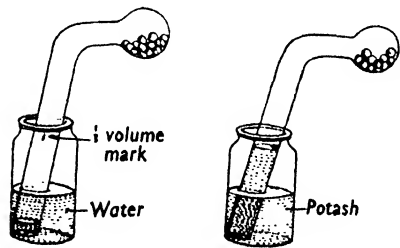


FIG. 68.—Diagram of experiment used to demonstrate gaseous exchange during respiration.

potash was added, it would appear that the volume of carbon dioxide given out by the peas was approximately equal to and not less than the volume of oxygen absorbed, and this will be found to be true of many respiring tissues. Seeds with oil as the main reserve usually absorb oxygen more rapidly than they give out carbon dioxide, and some succulent tissues, such as those of the cactus, may under certain conditions absorb oxygen for hours without giving out any carbon dioxide at all. On the other hand, some living organisms, of which the yeast used in baking and brewing is an example, will go on growing and giving off carbon dioxide for days in the absence of oxygen, whilst over short periods even germinating peas may be used to demonstrate this "anærobic" type of respiration. If germinating peas, preferably with their skins removed, are inserted over mercury in an inverted test tube, full

of mercury, the level of the mercury will soon be lowered by the production of a gas, which will be completely absorbed by potash. As the experiment is usually carried out it must remain open to question whether the carbon dioxide evolved is due to the metabolic activity of the peas or from the bacteria or other small organisms living on the surface of the peas.

In respiration the ratio of oxygen absorbed to carbon dioxide released seems to vary from $\frac{0}{1}$ to $\frac{1}{0}$ in different plants under different conditions, whilst in photosynthesis the ratio of carbon dioxide to oxygen released was uniformly $\frac{1}{1}$. In photosynthesis this was one of the few items of direct experimental evidence that pointed to the production of carbohydrates at an early stage in the process. It appeared that the metabolic changes in the process of photosynthesis always commenced by the formation of the same substances and that as a result the ratio of the gas exchange did not vary. Thus it appears that in respiration the opposite condition holds and that almost any type of chemical change may be concerned, as the result of which oxygen may or may not be absorbed, carbon dioxide may or may not be released. When a term covers such a wide range of metabolic activities as this we must inquire if it still retains any definite meaning, and indeed, with continued use of the word, its meaning has become continually less obvious. Respiration conveys to our minds first of all our action in inspiring and expiring air into and out of our lungs. This is merely the first stage in the true process of respiration in our bodies, and we realise now that a more significant phase of the process takes place in our tissues when the oxygen carried to them in the blood is absorbed and carbon dioxide is released. In the plant the gaseous diffusion providing for the absorption of oxygen and the dissipation of the released carbon dioxide is proceeding quietly all the time, first between the huge water-air surface of the intercellular system, when the carbon dioxide comes out of solution and the oxygen dissolves, and then by gaseous diffusion between this intercellular air and the external atmosphere. Here there is no tendency to identify the actual process of respiration with any direct air movement, but it is difficult to characterise the multifarious chemical changes which proceed differently in different cells and which really make up the process of respiration.

In photosynthesis energy has been stored in the formation of various organic compounds, derived originally from carbon dioxide and water. In every living cell the maintenance of life, and still more of active growth, necessitates continually that work should be done and energy expended, and this energy must be obtained by breaking down these compounds again to carbon dioxide and water; to these breaking down processes by which energy is released we intend to apply the term respiration, but we are so vague as to what the processes actually are that we cannot apply the term with great exactitude.

Usually the processes involve the absorption of oxygen and evolution of carbon dioxide, just as when similar organic substances are burnt, and further the same amount of energy is ultimately released in the process as would be released as heat, if the same substance were burnt under conditions in which all the energy was transformed into heat. This heat energy is measured in calories, and we measure the values of substances used as food in terms of calories; but this does not imply that those substances are burnt in the usual sense of the word, or that all the energy is transformed into heat. In plant tissues, as in the animal, the process of oxidation is a much slower one than in the case of ordinary burning; it takes place between substances in solution in water, and most of the energy is liberated in forms other than heat. In a warm-blooded animal of course, the temperature of the body is maintained above that of the surrounding air by these respiratory processes, but in the plant, and probably associated with its much larger bulk of water in proportion to living protoplasm, it is not usual to notice any rise in temperature in the respiring organism. A rise in temperature may, however, be demonstrated very simply in germinating peas or in opening flower buds if these are placed in a thermos flask. The vacuum between the double glass walls of the flask prevents the rapid dissipation of the heat, and under such conditions quite a considerable rise in temperature may be recorded. The experiment may be made more instructive if it is elaborated in the following manner: four thermos flasks are taken and these are filled about half full with peas, packed in amongst damp cotton wool. A thermometer is inserted, and the neck of the flask is plugged with

dry cotton wool. The peas in one flask are previously killed by boiling and are then sterilised by soaking in a solution of mercuric chloride. Into another, boiled and cooled peas are placed without the subsequent sterilisation. Into the third and fourth flasks soaked peas are placed, and one flask is held in the usual erect position, whilst the other is inverted. In order to reduce the growth of bacteria and other micro-organisms on the living peas, it is advisable to wash the dry peas in mercuric chloride solution and then to soak them in a solution of potassium permanganate. The following temperatures were recorded in such an experiment :—

TABLE 9.
TEMPERATURES OF PEAS IN THERMOS FLASKS.

Day.	Peas boiled and sterilised.	Peas boiled.	Peas living.	Peas living. Flask inverted.
	° C.	° C.	° C.	° C.
Wed. 9.0 a.m.	16.5	16.5	18.0	19.0
„ 6.30 p.m.	17.0	17.0	21.0	22.0
Thurs. 9.0 a.m.	17.0	17.0	26.5	27.5
„ 6.30 p.m.	18.0	18.0	30.0	31.5
Fri. 9.0 a.m.	17.5	19.0	29.5	30.0
„ 6.30 p.m.	18.5	22.0	29.5	30.0
Sat. 9.0 a.m.	18.5	31.5	27.0	27.5
„ 6.30 p.m.	18.0	38.0	24.5	25.5
Sun. 9.0 a.m.	15.5	51.0	17.5	19.0
„ 6.30 p.m.	14.5	52.0	17.0	17.5
Mon. 9.0 a.m.	15.0	52.0	17.5	18.5
„ 6.30 p.m.	16.5	53.0	20.5	21.0
Tues. 9.0 a.m.	17.0	51.5	20.0	22.0

The dead sterilised peas act as a control, and show the slight degree of fluctuation of the temperatures inside the thermos as caused by the variations in the room temperature. Thus the fall on Sunday morning is due to the lower laboratory temperature during the week-end, an effect which is only recorded relatively slowly in the flask.

The rapid rise of temperature in the putrefying mass of boiled peas suggests that, even after most of the oxygen has been used and the air around the peas is charged with carbon dioxide, respiration, and as a result release of energy as heat, is going on with astonishing rapidity. This effect,

however, does not appear until after the second day and is not very marked until the fourth day. The effect in this flask is due to the activity of minute anærobic organisms which grow and multiply on the dead peas very vigorously under the conditions of the experiment. Their destructive metabolism may increase in rate with rise in temperature so rapidly, that reactions may be set going which continually accelerate with rising temperature, independently of the life of the organisms responsible for their initiation. Changes of a similar kind may be caused by anærobic organisms at the centre of a rick of damp hay, until the decomposition of the organic materials at the centre of the rick is proceeding at a rate which releases sufficient heat to set the rick smouldering. If a rick in this condition is opened up sufficiently early, the entry of air actually hinders the activity of these anærobic organisms and the temperature falls, but if this action is delayed too long, the smouldering rick may burst into flames.

Comparing the records for the living peas with that of the dead, it is seen that the rise is initiated earlier and reaches its highest point at the end of the second day, that is, before the putrefaction effect is evident in the boiled peas. Beyond this the temperature gradually falls again and finally is only a little above that of the control flask. The facts suggest that the rise in this case is due to the respiration of the peas themselves, and this is supported by the appearance of the peas at the end of the period, when they are seen to have germinated well. The cessation of growth appears to be due to insufficient moisture.

For the fourth flask the record is very similar, but the temperature rise is slightly earlier and rises slightly higher. This effect is due to the fact that carbon dioxide is rather heavier than air, so that with the inversion of the flask this does not accumulate to the same extent in the flask and the peas make better growth.

If any chemical reaction which may release energy for growth or for the maintenance of vital activities is to be classed as respiratory in nature, it is evident that respiration is a very wide term. One American physiologist has suggested that we should use "energesis" instead, but it is questionable if anything is to be gained by multiplying terms or revising definitions until we have a better understanding of the whole subject.

Within recent times the activities of the oxidising enzyme systems of certain plants have been under examination and considerable light has been thrown upon the probable course of respiration in such plants ; at the same time this recent work has emphasised the fact that the enzyme systems differ from one plant to another so that no generalised scheme of respiration, applicable to all plants, can be formulated at the present time.

As an introduction to the study of the oxidising enzymes it is useful to consider one way in which it may be demonstrated that a specific chemical change in the tissues of a living plant occurs in the presence of an enzyme system, the action of which is under cytoplasmic control. In the living leaves of cherry laurel (*Prunus Laurocerasus* L.) there is present a glucoside, that is a substance yielding glucose amongst its products of hydrolysis ; if the leaves are crushed, hydrolysis of the glucoside takes place in the presence of an enzyme and the three constituent substances, glucose, benzaldehyde and hydrocyanic (prussic) acid are liberated. Prussic acid is gaseous and very poisonous and it is for this reason that the insect collector may prepare his killing bottle simply by placing some well-crushed cherry laurel leaves in a stoppered bottle. Filter paper soaked in an aqueous solution of equal parts by weight of sodium carbonate and picric acid provides a very delicate test for prussic acid, and a strip of such paper, yellow at first, soon turns brick-red when suspended over crushed cherry laurel leaves in a closed bottle. If strips of sodium picrate paper are suspended in four different closed bottles over (1) a normal fresh cherry laurel leaf, (2) a well-crushed leaf, (3) an intact leaf in the presence of a little cotton wool moistened with chloroform, and (4) a leaf killed by immersion in boiling water, the paper will turn red in a few hours in bottles (2) and (3). In these two cases, due either to the mechanical injury or to the anaesthetic, the semi-permeability of some or all the cells has been destroyed, the enzyme and the glucoside have met and prussic acid has been released. In (1) the cells are alive and intact, the cytoplasm retains its semi-permeability and no reaction has taken place. In (4) it is true that glucoside and enzyme have been released by the death of the protoplast, but the heat has destroyed the enzyme. Most plant enzymes either are proteins or work only in conjunction with proteins and when

the protein is coagulated by heat the enzyme is no longer able to catalyse the reaction. This experiment with cherry laurel leaves may have practical value in later years to some student whose work has taken him to less explored lands, where cases of poisoning of stock not infrequently occur and where death has apparently been due to the grazing of the animals on some unknown plant. The production of prussic acid from crushed plant tissues is not a very rare occurrence and may be one explanation of the trouble. If a few small corked tubes containing strips of alkaline picrate paper are carried in the pocket, a few leaves may be crushed and placed in these tubes and the test described above will soon show whether any suspected plant has a tendency to give off prussic acid.

Oxidising Enzymes.

In many plants injury leads to discoloration of the tissues as the result of the oxidation in the presence of air of substances allied to the tannins ; such discolorations are commonly observed at cut surfaces of potato and apple and attention has already been drawn to the blackening of injured tissues of broad bean. An alcoholic extract of guaiacum resin (derived from the woody plant *Guaiacum officinale* L.) provides a brown solution and a filter paper moistened with this turns a faint blue in air due to oxidation but only after long exposure. If a similar filter paper is held for a moment between two freshly cut surfaces of a potato tuber, the paper rapidly turns a bright blue indicating much more rapid oxidation. The oxidising action, judged by the intensity of the blue colour, is particularly active a short distance beneath the skin. That an enzyme released from the cut cells is responsible for this effect is suggested by an experiment of the same type as that carried out on the cherry laurel leaves. If a potato is cut into pieces and put in a bottle with cotton wool moistened with chloroform, the tissues retain their power to oxidise the guaiacum solution and the cut surfaces also discolour more rapidly than before ; the chloroform kills the protoplasts and the resulting loss of semi-permeability releases the enzyme from intact cells below the cut surface, whilst the activity of the enzyme itself is unaffected. If, however, the potato is thoroughly steamed, the tissues no

longer give the blue colour with guaiacum or discolour in air, as the enzyme has been destroyed.

The enzyme may easily be extracted. Thick slices of potato tubers are minced, placed in a bottle which is filled up with water to which a few cubic centimetres of chloroform have been added and the bottle is stoppered to exclude air. After standing for a few hours or overnight, the liquid is strained through a cloth and will be found to turn guaiacum extract blue very rapidly. If some of this potato extract is filtered and allowed to run slowly into strong methylated spirit (rectified, 95 per cent. alcohol) in such proportions that the final solution is between 60 and 70 per cent. alcohol, the oxidising enzyme, doubtless along with other substances present in the water extract, has less affinity for water than alcohol and is precipitated as a flocculent, greyish mass. This precipitate can be collected and dried and, though by no means a pure preparation of the enzyme, upon redissolving it will again give the oxidising action with guaiacum, or if added to a solution of pyrogallic acid it will readily bring about a purple discoloration and then the precipitation of purpuro-gallin.

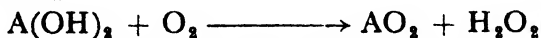
Enzyme systems which are responsible for direct oxidations of this kind are described as oxidases and their presence can be demonstrated with the guaiacum reaction in over half the families of flowering plants. Where discolorations of wounded tissues also occur the recognition that these are due to enzyme action has some practical significance. Thus the latex which issues from the wounds in rubber and other latex-containing plants often discolours on standing and this effect is usually due to such oxidases; when this fact was recognised it led immediately to methods of control in industrial processes, both in the rubber industry where a minimum of discoloration is desirable, and in the lacquer industry which depends upon coloured products such as lacquer varnishes.

In the other 50 per cent. of families of flowering plants cut surfaces of the tissues in air do not possess the property to blue guaiacum directly, but if the cut surface is moistened with hydrogen peroxide blueing results and the plant is said to possess a peroxidase system. This reaction may be demonstrated on pieces of horse-radish root (*Cochlearia Armoracia* L.)*; the thinner pieces of root prove the more satisfactory and the greatest care should be taken to use clean instruments which

* *Amoracia lapathifolia* Gilib.

have not already been in contact with tissues of plants containing the direct oxidase system. The occurrence of plants with the peroxidase system again emphasises that fact that the enzyme systems present and the course of the oxidations will probably prove to vary widely from one case to another.

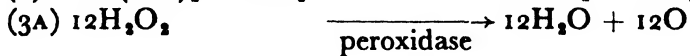
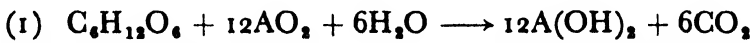
There is thus no difficulty in linking the colour changes of tissues, or of guaiacum extract by such tissues, in air with the presence of oxidising enzymes and it is an easy step to the idea that these enzymes are involved in the various oxidative processes that we collectively call respiration. The difficulty that discoloration does not normally occur in uninjured living tissues will be seen later to be apparent rather than real, and the fact that the sugars do not readily oxidise in air at room temperatures, as does the guaiacum extract, is of no moment in view of the obvious potency of the oxidase systems. Even in plant tissues it appears to be of rare occurrence for sugars to become oxidised directly to carbon dioxide by addition of oxygen to the sugar molecule. In the plants which possess the oxidase system, evidence has accumulated to show that sugar, or more correctly products formed from a preliminary breakdown of sugars, are oxidised not so much by the direct addition of oxygen as by removal of hydrogen from the molecule. The hydrogen can be removed from the sugar if some other substance is present in the system to pick up the hydrogen and it is in this connection that the tannin substances and their associated enzymes come into the story. Tannin substances are extremely common in plants, some of them entering into the composition of the red, blue and some of the yellow pigments of plants, and many of these substances contain the catechol grouping—a benzene ring with two hydroxyl groups ; in the presence of the suitable enzyme the catechol is oxidised by the removal of the hydrogen of the hydroxyl groups and the hydrogen removed is combined with oxygen to form hydrogen peroxide or water. This reaction we may picture simply by representing the benzene ring (and the rest of any tannin complex to which it may be attached) as A



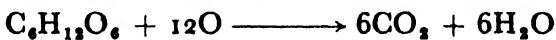
The enzyme concerned produces this oxidation by removal of hydrogen from the tannin and is therefore described as a dehydrogenase (or less aptly as a dehydrase), the substrate for the action of the dehydrogenase is the tannin compound and

the two together constitute the oxidase system. In injured tissues in the presence of air the reaction progresses in the direction of the arrow and the oxidation product AO_2 accumulates and accounts for the colour changes of natural tissues (or the blueing of guaiacum if this is present). The compound AO_2 is a powerful oxidising agent as it readily picks up hydrogen again and returns to the A(OH)_2 form ; it cannot recombine with the hydrogen removed by the dehydrogenase as that is now combined with oxygen, so that in living tissues hydrogen is removed from sugars. The tannin substance is continually undergoing these successive oxidations and reductions so that in living tissues discoloration does not occur.

Oxygen is essential for this type of respiration as it combines with the hydrogen removed from the tannins and such respiration is described as *aerobic*. A further stage in the process is concerned with the breaking down of the hydrogen peroxide, since this would be toxic to the tissues if it accumulated. An enzyme known as a peroxidase is involved here which breaks down the hydrogen peroxide into water and atomic oxygen, which probably completes the oxidation of the already partly oxidised sugar to carbon dioxide and may also effect other cell oxidations, or alternatively the specific enzyme catalase may break down the peroxide into water and molecular oxygen. These reactions may be expressed in general equation form as follows :



If the reaction in (3) produces nascent oxygen as in (3A), then we may have the additional reaction

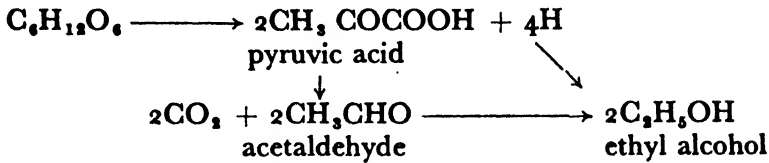


In either case the equivalence between oxygen absorbed and carbon dioxide released when sugar is the substrate for respiration is explained. The whole system is complex but amounts in essence to the removal of hydrogen from sugars and the

handing along of the hydrogen from one compound to another, and finally to oxygen, to ensure that the reaction proceeds in the direction of continued oxidation. Kermack and Eggleton in their book *The Stuff We're Made Of* draw an analogy between these processes and two travellers trying to get across a river too wide for them to jump by making use of stepping-stones ; two such stepping-stones are provided by the enzyme system. The two hydrogen atoms jump from the original breakdown product of the sugar from one stepping-stone to another and finally to the oxygen molecule forming hydrogen peroxide. They point out that this device of carrying out a difficult reaction in a relatively large number of quite small stages is indeed characteristic of many processes of the living cell.

The respiratory system thus far described involves the presence of oxygen as the final acceptor for the hydrogen, but the limited germination of peas over mercury and accompanying release of carbon dioxide shows that peas can respire for a time without access of oxygen. *Anaerobic* respiration of this kind is the normal type of respiration of many types of yeasts and bacteria, some of which are able to live only in the absence of oxygen. As the oxidation of the sugars or their breakdown products in aerobic respiration is normally initiated by the removal of hydrogen from the substrate, it is obviously possible that this process might continue under anaerobic conditions provided that some other substance can serve as the final hydrogen acceptor in place of atmospheric oxygen. Even in the normal aerobic processes we have good evidence that the sugar is partially broken down by enzymes (other than the oxidative enzymes), which work in the presence of phosphates, and amongst the breakdown products are certain organic acids (pyruvic and lactic acids). Reference to the chapter on the carbon atom will remind the student that organic acids were formed by oxidation of aldehydes and it is obvious that the reverse will also be true that reduction of organic acids will tend to give rise to aldehydes and incidentally to the still further reduced compounds, the alcohols. In the present case the breakdown products of the sugars, in the absence of the chain which would successively transfer hydrogen from them finally to oxygen, undergo changes whereby part of the molecule is oxidised fully to carbon dioxide and the rest is further reduced

to ethyl alcohol. These reactions may be represented simply, if somewhat diagrammatically as follows :



This series of reactions also evolves energy, but the energy released per unit weight of sugar transformed is obviously less than that produced in aerobic respiration since some is still tied up in the alcohol molecule. This is the type of respiration which occurs in the fungus yeast when it is growing at the bottom of a vat of fermenting malt liquor, when the product of industrial importance is the by-product alcohol. It is of some interest that many of the bacteria that normally grow under anaerobic conditions, and therefore always respire by this type of respiration in which hydrogen peroxide is not produced, do not contain the enzyme catalase ; if such organisms are exposed to air, oxygen behaves as the hydrogen acceptor and hydrogen peroxide is formed and accumulates and, being toxic, causes the death of the anaerobic organism. Fresh air is therefore an important factor in the treatment of diseases caused by anaerobic organisms.

The presence of a dehydrogenase may be demonstrated by the effect of potato tissue on methylene blue under anaerobic conditions. A relatively deep blue solution of methylene blue is placed in two glass cylinders and pieces of potato tuber are put in one, whilst the other is left as a control, and both are closed by glass plates. After standing overnight the dye in the neighbourhood of the potato pieces becomes decolorised, whilst that in the control cylinder remains unchanged. In this experiment the potato is well beneath the solution and has no access to free oxygen, but the methylene blue serves as a hydrogen acceptor and in the reduced form loses its blue colour.

The presence of catalase may be demonstrated by the liberation of oxygen from hydrogen peroxide. Pieces of potato tuber are simply dropped into a test-tube of hydrogen peroxide and rapid bubbling off of oxygen indicates the presence of catalase. The rate of evolution of oxygen may be compared when the piece of potato is (a) fresh, (b) previously treated with

chloroform, when the evolution will be equally or even more vigorous than with fresh potato, and (c) boiled, when no evolution of oxygen occurs owing to destruction of the enzyme.

In these systems of respiration it is obvious that numerous reactions are involved and that each has its specific enzyme. It appears that the active part, or prosthetic group, of these enzymes is a complex containing a metal ; some of the peroxidase enzymes for instance are complex organic substances, containing iron, built up around the same fundamental atom grouping haem, as occurs in chlorophyll and the red blood pigment haemoglobin. Haemoglobin differs essentially from chlorophyll in containing iron in place of magnesium and this difference is associated with the different function of the former which is to carry oxygen to the tissues of the animal. It is particularly interesting to find related iron-containing compounds in plants associated with the respiratory mechanism and the release of oxygen in a form to bring about final oxidations in the cells. Some of these peroxidase substances have recently been identified with some of the essential vitamins of animal nutrition which suggests that, as in the case of some amino-acids, the animal has to have these substances ready manufactured from the plant. Both the haem compounds of plants and the haemoglobin of blood are able to blue guaiacum in the presence of hydrogen peroxide, but the plant enzyme works in conjunction with a protein which is unstable to heat so that the capacity to produce the blue colour is lost on heating, whilst the protein attached to haem in haemoglobin is more stable to heat and will still blue guaiacum after steaming ; this has proved a useful way to distinguish a blood stain from a vegetable stain on clothing, etc.

Finally it should be emphasised that all the reactions which contribute to the release of energy in respiration are taking place between substances in solution. The process of respiration is thus under protoplasmic control and its rate will be affected by the rate at which dissolved substances entering into the reactions meet one another and by the amount and nature of the enzymes present. The control afforded by the semi-permeability of the protoplasm is therefore a very important part of the mechanism which regulates respiration and indeed all metabolic change.

CHAPTER XXI.

EVAPORATION FROM THE LEAF.

THE vital activities of the leaf are closely connected with its environment, and from this standpoint it is clearly of importance that the leaf exposes a large surface to both light and air. In particular the preceding anatomical study has shown how very large must be the water-air surface of the leaf when its internal structure is considered.

Wherever a water-air surface exists an interchange of water molecules between air and water will continually be taking place. The physicist tells us that when the air is saturated with water vapour the same number of molecules of water enter and leave a pure water-air surface in any interval of time, but where the water contains substances with great affinity for water, more molecules of water will enter the surface from saturated air than leave it, so that the solution will increase in volume. On the other hand, when the air is not saturated, both pure water and solutions will usually decrease in volume owing to evaporation.

At the water-air surface in the plant we are never dealing with pure water and the content of water vapour in the air is always varying, so that the volume of water in the leaf may increase or decrease as the result of exchanges of water molecules at the surface. Usually the air is so far from being saturated that evaporation proceeds rather than condensation and the leaf loses water to the air, but under special circumstances leaves may absorb water from the air.

The evaporation of water from the leafy shoot is usually spoken of by the botanist as transpiration, a term which is used by the physicist in another sense, but its botanical usage should lead to no confusion if the fact is borne in mind that the physical process of evaporation is the basis of the phenomenon studied. Whether the water is lost from the internal

water-air surface of the intercellular space system and then through the stomata, or directly to the outside air through the cuticle, its loss forms part of the process of transpiration.

With such a large surface exposed, frequently in relatively dry air, this phenomenon achieves very considerable dimensions, and makes the leafy canopy covering the earth's surface a factor of importance in relation to atmospheric humidity, rainfall, and water supply. Estimates based upon short periods of observation may introduce very large errors, but the following figures may be quoted to give an indication of the scale of the phenomenon. Haberlandt is quoted by Pfeffer as estimating the loss of water from a single maize plant during the 173 days of its development at 14 litres, and from an annual sunflower plant during 144 days at 27 litres. The same authority quotes von Höhnel's estimate of the evaporation from 1 hectare (about $2\frac{1}{2}$ acres) of beech forest, of trees approximately 115 years old, at 2.4 to 3.5 millions of litres in the period between June 1st and December 1st. This estimate is based primarily upon an estimate of a daily loss from one tree of 75 litres, making allowance for the number of trees to the hectare and the diminished evaporation from single trees owing to crowded conditions of growth in the forest, but admittedly the estimate may be very wide of the mark. Though the water loss seems so enormous when thus estimated, it must be remembered that these losses may not represent very rapid evaporation from unit area of the large leaf surface involved. Thus Stephen Hales, one of the pioneers of experimental plant physiology, after determining by direct weighing that the maximum loss of water from a sunflower plant was 30 oz. in a twelve-hour day, calculated that this represented only a loss of $1/165$ cubic inch per square inch of leaf surface. In fact when the loss of water from the leaf is compared with the evaporation from a free water surface, the loss from the leaf, if measured over a considerable period of time, is always appreciably less. In the case of sunflower, Sachs has estimated the loss at only $1/23$ of that from an equivalent free water surface, an indication that the plant exerts some control over the process of evaporation.

It is obviously very desirable that the estimation of water loss should be as correct as possible, but actually the measurement of water loss from the plant, *under normal conditions*, is

attended by very considerable practical difficulties. Not until our anatomical studies have extended to both stem and root can we profitably consider the problem of the supply of water to the leaf, but it is clear that the conditions of supply may have the greatest importance in connection with the control of evaporation. When supply is in excess of demand, it is a usual experience that the rate of evaporation tends to increase. When the supply of water is below the rate of evaporation, factors come into operation which reduce the rate of evaporation; this phenomenon will be examined more fully at a later stage, at the moment these statements may suffice to show that the normal rate of evaporation can only be measured on the intact plant, not by a study of water loss from leaves separated from the plant. The most suitable method then would seem to be a measurement of loss from the whole plant, including the root system in soil, a loss which can be measured as loss in weight, since the changes in weight due to photosynthesis, respiration, etc., are negligible in comparison with the loss in weight due to evaporation. Obviously for such purposes the root system needs to be in a suitable container, such as a glazed, non-porous pot or metal vessel, and if the root system is to be allowed to spread in the soil without disturbance, then soil and plant together are so heavy that an expensive balance will be needed in order to register small changes in weight accurately. During the experiment precautions have to be taken to guard against water loss from the surface of the soil and pot, but with suitable precautions and the use of adequate balances, our most accurate estimates of evaporation from the surface of the shoot have been obtained by such methods. Veihmeyer and Hendrickson obtained the following values by the method of weighing whole rooted plants. The experiments were carried out under Californian conditions on French prune trees. The total water loss from four trees during the period 1st March to 25th September was respectively 499, 316, 1020, and 508 pounds. When this was worked out as loss per square inch of leaf area over the same period, the figures obtained were 0.237, 0.254, 0.237, and 0.245 pounds respectively.

Such methods are not available for ordinary laboratory class work, and attempts have been made to devise simpler methods which may still be used on the intact plant. Leaf

loss of water can be determined by weighing, and this rate of loss may be checked against the rate of absorption as shown by the fall in level of the water in the graduated container. Results obtained by such methods only have a relative value, but by their use it is possible, for instance, to show rapidly and simply that in most plants loss of water in darkness is much less than in light. Observations of the rate of evaporation with the potometer may also be carried out in association with observations upon the degree of stomatal opening by means of the porometer (Chapter XVI). Comparisons made under a variety of external conditions show that the loss of water by evaporation is usually controlled by the stomata, the degree of opening of which regulates the communication between the gas in the intercellular air space system and the external air.

Evaporation from External and Internal Surfaces Compared.

The external surface of the shoot, as we have seen, is covered with a continuous layer of fatty, varnish-like material, the cuticle. In effect the external surface is thus an air-cuticle surface, from which the loss of water to the air is much diminished by the small extent to which water dissolves in the cuticle. In older leaves the cuticle may become relatively dry and brittle and even crack, whilst in young leaves it is thinner, more liquid, and may contain more water, so that the water loss through the cuticle from very young or from older leaves may be greater than in the case of an ordinary adult leaf with a continuous unbroken cuticle.

The significance of the nature of the external plant surface in reducing evaporation is illustrated by the figures given in Table 10. The loss of weight from apples and potatoes, kept in an oven at a constant temperature of 25° C., is expressed in each case as a percentage of the previous weight. The loss in weight is mainly due to loss of water, and it will be seen that it is much greater from the peeled apple than from the unpeeled; from the latter the slight loss remains almost constant in value throughout the experiment. The peeled potato also loses at first at a high rate, comparable with that shown by the peeled apple, but after a few days the rate of loss falls off conspicuously in the potato, whilst it remains at a high value in the apple. The explanation of

this is that the original skin of the potato was a layer of cork (see Chapter XXV), and after a few days a new cork layer is regenerated at the surface of the peeled potato. No equivalent regeneration of new cuticle takes place at the surface of the peeled apple.

It is almost impossible to get a direct comparison of the loss through the external surface with that through the internal water-air surface of the intercellular space system, but a sug-

TABLE 10.

DAILY LOSS OF WEIGHT FROM NORMAL AND PEELED APPLES AND POTATOES.

(All specimens kept in constant temperature oven.)

Original weight.	% loss, calculated on weight on previous day.						
<i>Apple (Whole)</i>							
(1) 102.9	0.28	0.32	0.27	0.24	0.24	0.24	0.24
(2) 96.1	0.32	0.39	0.37	0.28	0.29	0.37	0.31
(3) 81.7	0.48	0.43	0.43	0.24	0.37	0.37	0.37
<i>Apple (Peeled)</i>							
(1) 87.6	5.77	9.13	10.73	9.14	9.71	12.18	12.30
(2) 67.8	5.60	11.78	13.49	13.55	11.53	13.52	14.55
(3) 80.5	4.92	9.33	11.03	13.45	12.36	14.00	14.78
<i>Potato (Whole)</i>							
(1) 158.3	0.31	0.24	0.26	0.19	0.19	0.19	0.19
(2) 138.7	0.47	0.48	0.42	0.40	0.37	0.36	0.36
(3) 92.5	0.44	0.46	0.40	0.32	0.38	0.38	0.30
<i>Potato (Peeled)</i>							
(1) 149.1	4.26	5.21	4.01	2.36	1.82	1.44	1.38
(2) 162.4	5.45	6.02	4.01	2.49	2.18	2.08	1.74
(3) 164.2	3.28	6.39	3.80	2.37	1.75	1.20	1.47

gestive comparison may be made between the loss of water from the two surfaces of a leaf in which stomata are practically confined to the lower surface. The loss from the lower surface will include some loss through the cuticle, which is usually thinner than on the upper surface, but if the loss is very markedly greater we may conclude that this difference is due in part to loss through the stomata. It is not difficult to find leaves in which stomata are restricted to the lower surface, e.g. rose, hawthorn, but it is by no means easy to devise a simple

experiment by means of which the water loss from the two surfaces may be compared. One simple method gives a good qualitative indication of the comparative rate of loss of water from the two surfaces. Cobalt chloride is blue when dry but turns pink as it takes up moisture; when, therefore, filter paper soaked in a solution of cobalt chloride is dried, it turns blue, but if left in the air it absorbs water and turns pink. A convenient method is to place a small circle of this paper over a linen eyelet hole (such as are used to reinforce the perforations in loose-leaf sheets of paper) and then to gum two of these on to a small strip of cellophane. The cobalt chloride paper is thus exposed through the eyelet hole, but is protected on the outer side by the cellophane. After thorough drying in a desiccator, the cellophane is folded with the eyelet holes inwards and fastened over the margin of a suitable leaf (e.g. *Pelargonium*) with a wire paper clip, so that one circle of cobalt chloride paper faces each leaf surface. The change in colour can then be seen through the transparent cellophane. If the stomata are present mainly on the lower surface and are open, the paper changes colour much more rapidly over this surface than over the upper, so that this experiment suggests that normally, when the stomata are open, evaporation takes place very largely through the internal water-air surface and the stomatal aperture very effectively controls the process. On the other hand, we must not conclude that under all circumstances, and in all leaves, we can neglect the loss of water from the external surface.

Effect of Water Content of the Plant upon Rate of Evaporation.

As the water content of the leaf falls it is clear that the internal water-air surface will alter in configuration. So long as there is plenty of water, every protoplast will become fully turgid, for it will take in water and expand until it cannot extend the cellulose wall any more; if water is still available, it can then no longer be taken into the protoplasts, but remains in the walls around them or even begins to displace air in the intercellular spaces. We have not yet considered how the water enters the leaf but it may certainly be expected to enter by the veins. If leaves of plants growing in a greenhouse are held up to the light in the early morning,

clear translucent regions may often be seen around the veins, which indicates that water is injecting the intercellular spaces in these regions. Also in certain plants, such as *Tropæolum* or young seedlings of barley, in the early morning little droplets of water are frequently seen to have been exuded from the ends of the veins. Thus it is clear that under certain circumstances, water may be present in the leaf in excess, when it will saturate the walls around the protoplasts and water will evaporate from such wet walls as readily as from a water-air surface.

Under other conditions, especially when a leaf has been evaporating during many hours of sunlight, we may expect to find that water is leaving the leaf more quickly than it is entering, so that the water content is falling. When this is the case, all water will be withdrawn from the intercellular spaces into the protoplasts and walls, and subsequently the water-air surface will sink still lower and recede into the substance of the wall. The wall will tend to dry at its surface and at the same time substances may be deposited out of the receding water. Judging by the difficulty with which the air is displaced from the intercellular spaces when leaf sections are mounted in water (Chapter XVI), it seems probable that the intercellular spaces are bounded by comparatively dry cellulose surfaces, on which traces of fats, etc., have accumulated. The evaporation from such an air-cellulose surface will not be so rapid as from a simple air-water surface, since the water will only diffuse slowly outwards through the comparatively dry cellulose with its film of accumulated substances. Under the circumstances, and particularly since the stomata in such relatively dry leaves are often not fully open, the rate of evaporation may not be materially different from upper and lower surfaces even if all the stomata are present on the lower surface.

Considerations of this kind should always be borne in mind in any experiments in which water loss is studied on leaves removed from the plant and thus cut off from their normal water supply. The following type of experiment is sometimes carried out on account of the simplicity of the experimental procedure, though the results obtained are actually very difficult to interpret. Isolated leaves of relatively large size, and usually of some evergreen plant such as cherry laurel

or rhododendron, are vaselined on upper, lower, or both surfaces and weighed at intervals, so that the loss of water through upper and lower surfaces can be compared. The weights are expressed as percentages of the original weight so as to make allowance for the different sizes of the leaves.

In Table 11 the results from an experiment on rhododendron leaves is given. The leaves remain on the plant for

TABLE 11.

LOSS OF WEIGHT OF ISOLATED RHODODENDRON LEAVES, EXPRESSED AS A PERCENTAGE OF THE ORIGINAL WEIGHT.

(Horizontal lines, weights for different leaves, vertical rows at successive 24-hour intervals.)

	Treatment of leaf.								
	Vaselined both surfaces.			Vaselined upper surface.			Vaselined lower surface.		
Leaves one year old	6	6	9	9	17	18	11	20	27
	7	7	8	10	16	21	7	11	17
	1	1	2	10	16	22	7	11	15
	6	6	6	9	12	17	11	24	35
	3	5	5	13	19	25	7	11	17
	7	8	8	9	13	17	6	10	12
Leaves two years old	10	11	13	9	19	26	9	17	25
	6	7	7	23	33	41	5	8	11
	2	2	2	16	29	39	5	9	14
	1	1	4	23	35	42	7	16	23
	4	5	7	27	39	43	5	8	13
	0	0	0	8	16	25	9	18	27
Leaves three years old	1	3	4	23	39	50	10	18	26
	7	8	10	16	25	32	14	26	38
	12	14	19	17	27	36	13	25	34
	8	9	10	22	36	48	13	25	35
	7	9	10	16	26	37	17	31	41
	6	9	13	19	35	45	11	19	27

three years and the results for first, second, and third year leaves are given separately (as the experiment was carried out in April, "first year" leaves are in their second season). Evaporation is not completely prevented from the leaves vaselined on both sides and the rate of loss from these leaves varies little throughout the experiment. Taking the results as a whole, the loss is greater when the lower side of the

leaf is left unvaselined so that the stomata are not blocked, and this is most marked in the second year leaves. First year leaves lose rather less water than the older leaves, but no conclusion is possible in this case as to the effect of vaseline on upper and lower surfaces respectively. Stomatal control should presumably be at its best in the healthy first year leaves, rather than in the older second and third year leaves, so that the general conclusion would seem to be that this experiment has contributed little or no evidence of the efficiency of stomatal control in isolated leaves. It has merely shown that the water loss is greater from the lower than the upper side in older leaves, whilst the reasons for this still require analysis. The greater loss through the upper side from third year, as compared with second year leaves, is probably significant and may be due to the cracking of the old cuticle on the more exposed upper surface, as these leaves curled sharply as they dried, with the upper surface outermost.

The Concept of Xeromorphy.

When evaporation is in excess of water supply to the leaf, the leaf cells are losing some of the water which normally enables them to keep the cell wall fully distended. The wall is only very slightly elastic, so that the loss of water will not proceed far before the wall must either collapse or the protoplast must begin to withdraw from the wall, as the liquid supplies available no longer fill completely the internal volume of the cell. In thin leaves the loss of turgor (the name given to the state of the cells when distended by the pressure of the liquid contents of the protoplast) is immediately evident by their generally flaccid appearance, when they are said to be wilted. Tough evergreen leaves may lose all turgidity and reach the same condition as regards the water content of the protoplasts without any visible signs of wilting. Such conditions, if not excessively developed and only temporarily produced, may not have any permanently harmful effects, but in dry climates, wilting may easily persist so long, or be so severe, as to damage the leaf tissues beyond recovery.

With the recognition of the harmful effects of excessive evaporation, it is natural that a number of structural features often seen in leaves should have been interpreted as structural

devices which tend to reduce evaporation and thus to prevent its harmful effects. They have in particular been regarded as significant as adaptive devices in plants which are able to survive in dry habitats ; such plants are called xerophytes and any features which are effective in reducing evaporation are described as xeromorphic.

One of the most characteristic features of many plants of a xerophytic vegetation is a tough, leathery consistency of the leaves which is due to the thick walls of the epidermal cells, usually the presence of a thick cuticle and often to the presence of sclerotic elements in the mesophyll ; such leaves do not collapse under conditions of water deficiency when the mesophyll cells lose turgidity. The stomata on such leaves are often numerous and when these are open under conditions of favourable water supply, the rate of transpiration is high. When water supply becomes deficient, the stomata close and only cuticular transpiration is possible and at this stage various xeromorphic features such as thick cuticle, waxy coverings, etc., almost certainly contribute to the drought-resisting powers of the plants. Many of the so-called xeromorphic features however, need guarded interpretation, as for example that of hairiness of the leaf. It is true that under windy conditions the presence of hairs will reduce the rate of dispersal of the moister air diffusing from the stomata or from the cuticular surface, but at the same time they produce an enormous increase in the area of the external surface. The hairs on plant shoots develop from turgid, superficial cells of young organs, the outer walls of which extend into hairs under the pressure of the vacuolating contents and hairiness of this kind is a common feature of the young leaves of many plants such as *Æsculus Hippocastanum* L. *Fagus sylvatica* L. and *Tilia vulgaris* Hayne, though older leaves of the same plants may not appear conspicuously hairy since the hairs collapse or become widely spaced by cell enlargement. It is obvious that in such a case the young hair cells with thin cuticle and watery contents present so large a surface for evaporation that they are likely to increase rather than to reduce the rate of water loss. In true xerophytes the persistent hairs usually die and dry out and remain as a thick covering, as the epidermal cells do not expand much under the conditions of deficient water supply, and there is experimental evidence

that in winds of moderate velocity such hairy coverings do tend to reduce evaporation.

In some plants of the moorlands such as heath (*Erica* spp.), ling (*Calluna vulgaris* L.) and crowberry (*Empetrum nigrum* L.) the leaves are so curved that the lower surfaces bearing the stomata are almost completely enclosed in a groove. Recent experimental examination of the rate of water loss from plants of this kind suggests that evaporation is very vigorous when the water supply is adequate, so that the characters possessed by such plants do not function as adaptations to reduce water loss, at any rate under all conditions. At the same time they are probably structural features that are associated in some way with growth in a habitat where water absorption is sometimes difficult and evaporation rate high and they may be in this sense xeromorphic. It would appear true for most plants that when the leaf cells are fully turgid, the greater proportion of the water loss takes place from the internal water-air surface and is very effectively under stomatal control. After stomatal closure a number of xeromorphic features may contribute to the control of water loss as the water table in the leaf tissues falls, but this latter feature in itself is probably one of the most important as in many cases the evaporation rate has been found to fall markedly under dry conditions without any adequate reduction of stomatal aperture. Obviously the whole question of the water balance of the plant is a very complex problem and the rate of evaporation is closely bound up with the conditions of supply. A much fuller discussion of the points involved will be found in Maximov's book *The Plant in Relation to Water*. The question of the water balance of the plant should be reconsidered after the structure of the stem and root have been examined (Chapter XXIX).

CHAPTER XXII.

STEM STRUCTURE IN THE HERBACEOUS DICOTYLEDON. I.

IN the account of leaf structure in Chapter XIX little attention was paid to the vein system because this is continuous with a similar structural feature in the stem, where it is more conveniently examined. No part of the mesophyll of the leaf is far from a branch of the intricate network of veins in the lamina ; the finest branches link together into larger veins, which again unite into still larger strands until ultimately one or more are continued through the petiole into the stem. We have already noted the small dots which are left on the leaf scar by the ends of the veins.

If a leaf is held up to the light the veins appear as relatively translucent lines in the otherwise opaque leaf tissue and this is true even of the larger veins, though these are thicker than the rest of the lamina. Opacity in plant tissues is frequently due to the presence of intercellular spaces containing air, amongst the water-filled cells, so that evidently such air-filled spaces are absent or less developed around a vein. In a leaf the vein is separated from the mesophyll of the lamina by a ring of closely fitting cells, the bundle sheath, within which the cells are closely packed and intercellular spaces absent or very small.

If the base of a leaf, still attached to the plant, is cut across under an aqueous solution of the dye Magdala red, the solution is sucked into the plant and, as the dye travels in the veins without diffusion into the other tissues, it is a very simple method of showing the course of the veins from any particular leaf. A convenient plant for this purpose is wallflower (*Cheiranthus Cheiri* L.), in which only one vein enters the stem from each leaf. A transverse section of the petiole shows the

single, red-stained vein and a section of the internode of the stem below the leaf insertion shows the continuation of the same vein, which is now seen as one of a ring of similar veins. Sections of lower internodes may still show the injected vein which may run vertically down through five or more internodes. The ring of veins or vascular bundles, which is such a conspicuous feature of the wallflower stem, may then be regarded as built up of vascular bundles, which, since they are common to leaf and stem, are described as leaf trace bundles. The other veins of the ring are obviously related in the same way to leaves higher on the stem than the one cut under the dye. It is sometimes possible to see that the dye travels in the part of the vein directed towards the upper side of the leaf, since an uninjected portion of vein tissue is also visible towards the lower side; in the stem the injected portion is directed towards the centre of the stem.

As the same bundle is continuous in leaf and stem, its detailed structure can be examined in the stem. It is again desirable to adopt methods which will permit the construction of a three-dimensional picture of the structures examined. In the case of the herbaceous stem this is readily done by examining, in addition to transverse and longitudinal sections, other longitudinal sections or dissected tissues which have been macerated for about twenty-four hours with 5 per cent. chromic acid. Such treatments dissolve the calcium pectate and other substances which form the cement of the middle lamella, and some of the tissues readily separate into their component cells and lie in all directions in the preparation when the cover slip is gently tapped. Such methods can be applied with success to most plants, but in all cases it is essential to use fresh material. Different tissues are better developed and more readily studied in different plants, so that in this preliminary study of the herbaceous stem we will consider the structure of a fully extended internode of the annual sunflower (*Helianthus annuus* L.) in transverse section, and then as the various tissues are examined more closely, reference to other stems will be made in order to bring out the characteristic features of particular tissues. The general distribution of the tissues of the stem is indicated in plan in Fig. 76, and then a few cells of each tissue are represented as seen in both transverse and longitudinal radial section under the high power in Fig. 71. This

method of representing the results of a microscopic examination is strongly recommended to the student. The low-power diagram, showing the distribution of the tissues, is on too small a scale to permit the accurate representation of the cells in the tissues, and it is a waste of time to attempt to indicate the cells in this drawing. Similarly in the high-power drawing, the representation of a few cells with accuracy means time better spent than if many cells are roughly sketched in, in order to make the representation of a particular tissue more complete.

In the study of the distribution and detailed structure of the tissues of the stem, two points of view will be held in mind. In the first place the structures will be considered in relation to the story of their development, and then some attempt will be made to elucidate the connection between structure and function.

The general distribution of the tissues is the first point to examine. The leaf trace bundles are seen to lie roughly in a ring (Fig. 76) embedded in a ground tissue of parenchyma, which within the ring is known as pith, and without, as cortex. On the outside the cortex is bounded by the epidermis. This general arrangement is determined in the growing shoot, soon after the apex has given rise to a new leaf primordium. It will be remembered, from the study of the shoot apex of privet, that the leaf primordium grows more vigorously than, and soon overtops, the shoot apex, and at first, whilst it is still meristematic, the leaf insertion is not separated from the insertion of the next older leaf initial by any internode. As soon as vacuolation begins in the leaf primordium, the future vascular strands appear as eu-meristematic procambial, or desmogen, strands (Fig. 70a), which are left amongst vacuolating parenchyma. This differentiation is proceeding at the same time as the development of the associated internode below, vacuolating tissues and procambial strands of primordium and axis are continuous, and so the vacuolating cell system of the cortex develops in association with that of the lower surface of the leaf, whilst that of the pith is continuous, through the leaf gap in the procambial ring, with that of the upper surface of the primordium. Thus the future vascular system of the axis differentiates in a procambial meristem which tends to be a cylinder, but which is continually broken by the leaf gaps left by the procambial strands of successive leaves

(Fig. 70*b*). The rapid expansion of the internode and leaf is brought about mainly by the repeated divisions of the vacuolating and dividing cells and their continued expansion, which is often mainly in length. The procambial meristem cells grow mainly by the manufacture of new protoplasm, but lying as they do between two regions of vacuolating tissue, they tend to become longer. Such an increase in length means increase in mass with little or no decrease in proportion of surface to mass and this may be why these cells are able to grow larger than

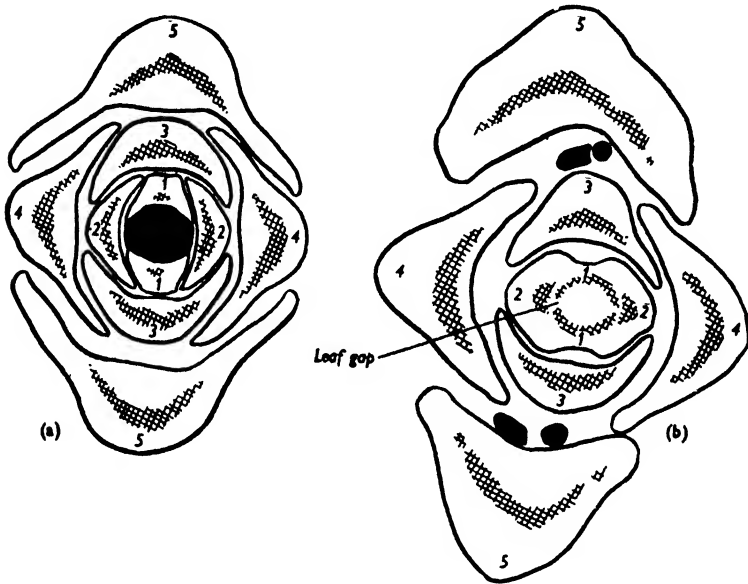


FIG. 70.—Plans of transverse sections of the shoot apex of privet to show the distribution of the eu-meristematic tissue (black) of the apex and of the future vascular tissue (shaded) ($\times 25$). (a) Below the insertion of the first pair of primordia, (b) below the insertion of the second pair of primordia.

the eu-meristem cells of the apex before they divide. The planes of division of procambial cells are determined in part by the growth of the organ in which they lie, but from the first they grow faster than the surrounding tissue and their first divisions are usually longitudinal; where they lie in rapidly elongating internodes, the procambial cells grow longer and undergo transverse divisions (Fig. 54*d*).

Thus the main features of tissue distribution in the stem are determined early in development. When vacuolation takes place, the exposed face of the epidermal cells is already

covered with cuticle and the cement between the anticlinal walls has become hardened by its exposure to air. This layer therefore forms a continuous skin, which is only interrupted by the pores between the guard cells of the stomata. Within the epidermis lie the vacuolating tissues that form the cortex, which extends in as far as the cylinder of procambial cells, from which develop later the leaf trace system of vascular bundles ; within the procambial ring lies the central pith, derived from tissues which vacuolate relatively early and which ultimately consists of parenchyma with a well-developed intercellular space system. We may now proceed to make a detailed study of the various tissues of the sunflower stem under the high power of the microscope, commencing with the epidermis.

Epidermis, Cortex, and Starch Sheath.

In the transverse section of the *Helianthus* stem the epidermis is seen to consist of somewhat flattened cells, which fit closely to one another along their radial walls (Fig. 71). The cells are curved on the free outer face and angular where they abut upon the sub-epidermal layer. In this view the cells vary considerably in size and especially in tangential dimensions, but on the whole the tissue is very uniform, apart from the presence of occasional stomata and massive hairs, which grow out from a group of epidermal cells.

In the central region of the cortex the cells appear large and rounded in this transverse view and are associated with conspicuous intercellular spaces. From the central region the cells tend to become slightly smaller towards the vascular ring and the intercellular spaces also decrease in size, until ultimately the innermost layer of cortical cells, which abuts on to the smaller cells derived from the procambium, forms a closely fitting, practically uninterrupted sheet. This layer has the characteristic that starch grains are almost invariably present in the cells, even when they are absent from other cells of the cortex ; it is therefore described as the starch sheath. In the lower internodes of many plants, including *Helianthus*, the cells of this layer lose their starch grains and undergo a different type of development to become an endodermis ; as the endodermis is a constant feature of roots its features will be described in connection with root structure (Chapter XXVIII).

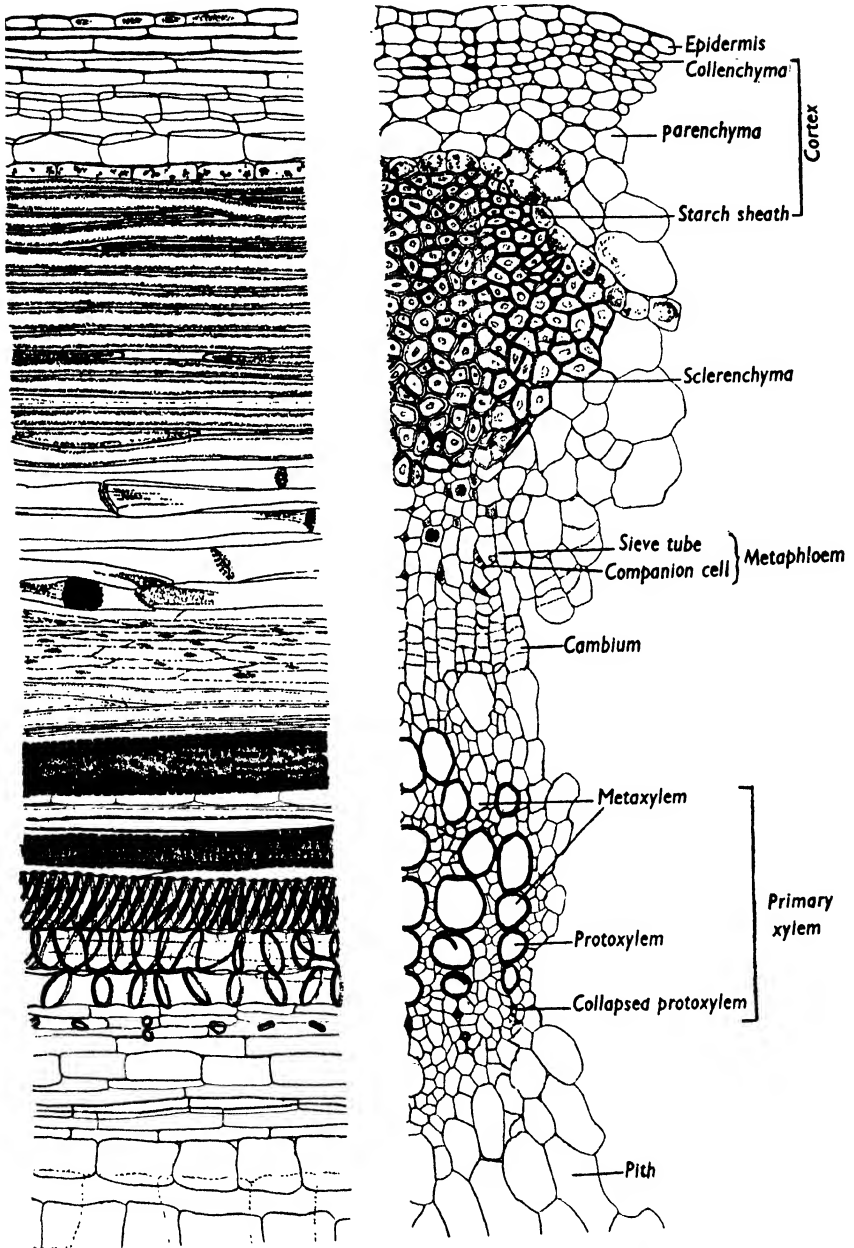


FIG. 71.—*Helianthus*. Fully extended internode in transverse and approximately radial longitudinal section ($\times 100$).

Towards the periphery of the cortex the decrease in size of the cortical cells, and their accompanying intercellular spaces, is especially marked; the cells are radially compressed, may undergo some radial divisions and have thicker tangential walls. Protoplasmic contents are present in the cells and these contain a number of plastids. In places in the cortex a ring of small cells may be observed to surround a small intercellular space; these are secretory canals which occur in many plants of the family Compositæ to which the sunflower belongs.

This tissue distribution in the cortex is readily understood in the light of development. Vacuolation commences in the central region of the cortex, so that here the cells round off early, developing an intercellular space system. At this stage they divide repeatedly in the transverse direction and so give rise to long files of relatively short, cylindrical cells, which constitute typical parenchyma. Outside the region of early vacuolating, middle cortex the cells remain meristematic longer and become radially compressed and vertically elongated owing to the pressure of the expanding tissues within. When these outer cells eventually vacuolate, the intercellular spaces between them are naturally smaller. In these smaller spaces the sap would linger, after it had been displaced by air from the larger spaces amongst the larger cortical cells. The walls of the outer cortical cells are often markedly thicker than those of the cells farther in, but the most striking feature is that the wall thickens, particularly where it borders upon an intercellular space. Whilst the central region of the cortex consists of typical parenchyma, the outer tissue with the characteristic thickenings of the walls at the angles of the cells is known as collenchyma (Fig. 71).

The transverse view of the cortical tissues should be supplemented by examination of material of the sunflower stem macerated in 5 per cent. chromic acid and stained in Heidenhain's hæmatoxylin and mounted in glycerine. In material treated in this way the epidermis remains in small sheets, as the cells are held together by the overlying cuticle. A sheet of epidermis seen in surface view shows the angular, slightly elongated, closely fitting cells to be arranged in short longitudinal files. If one of these sheets has fallen with the outside uppermost, it will be seen that fine folds of cuticle run the length of the cells, an effect which is due in part to the greater

contraction of the cells than the cuticle during the treatment, and for the same reason faintly grey-stained sheets of cuticle may often be seen free from the epidermal cells. The contents of the epidermal cells are seen as contracted protoplasts with a more deeply stained nucleus and numerous small plastids (Fig. 72a).

In the same preparation the tissues of the cortex are present, the most conspicuous of which are the relatively large parenchyma cells. These cells tend to remain in files of about five or six cells, though many of the cells are lying detached. The cells appear in this view slightly elongated or nearly square and must therefore be short cylinders, each with a contracted protoplast with nucleus and plastids (Fig. 72b). In the tissue, the intercellular spaces run vertically between the files and connect transversely at the constrictions, where the cells of the file tend to round off from one another in the region of a cross wall. There is considerable range in the relative length and width of the parenchyma cells, according to their position in the cortex, and there is a transition from the narrower parenchyma cells to the collenchyma. Amongst macerated material of the inner cortex the starch sheath will be recognised, because the cells of this layer do not readily fall apart from one another but tend to remain in small sheets. They are not held firmly together by a common sheet of fatty material over the surface, but, like the epidermis, the cells in tangential longitudinal view form a continuous pavement with practically no intercellular spaces between them, especially where they overlie vascular tissue. The radial and transverse walls of neighbouring cells are thus continuously in close contact and they appear to have fatty material dried into them so that they are not readily separated by oxidising agents. The individual cells are rectangular in this view and are particularly conspicuous when, as is normally the case, they contain numerous starch grains (Fig. 72c).

In unstained transverse sections the wall thickenings at the angles of the collenchyma cells are very conspicuous as they are white and refractive and may completely fill the regions where intercellular spaces would otherwise be present. They show even in young internodes as the thickenings develop very early, and indeed are less conspicuous in old woody internodes.

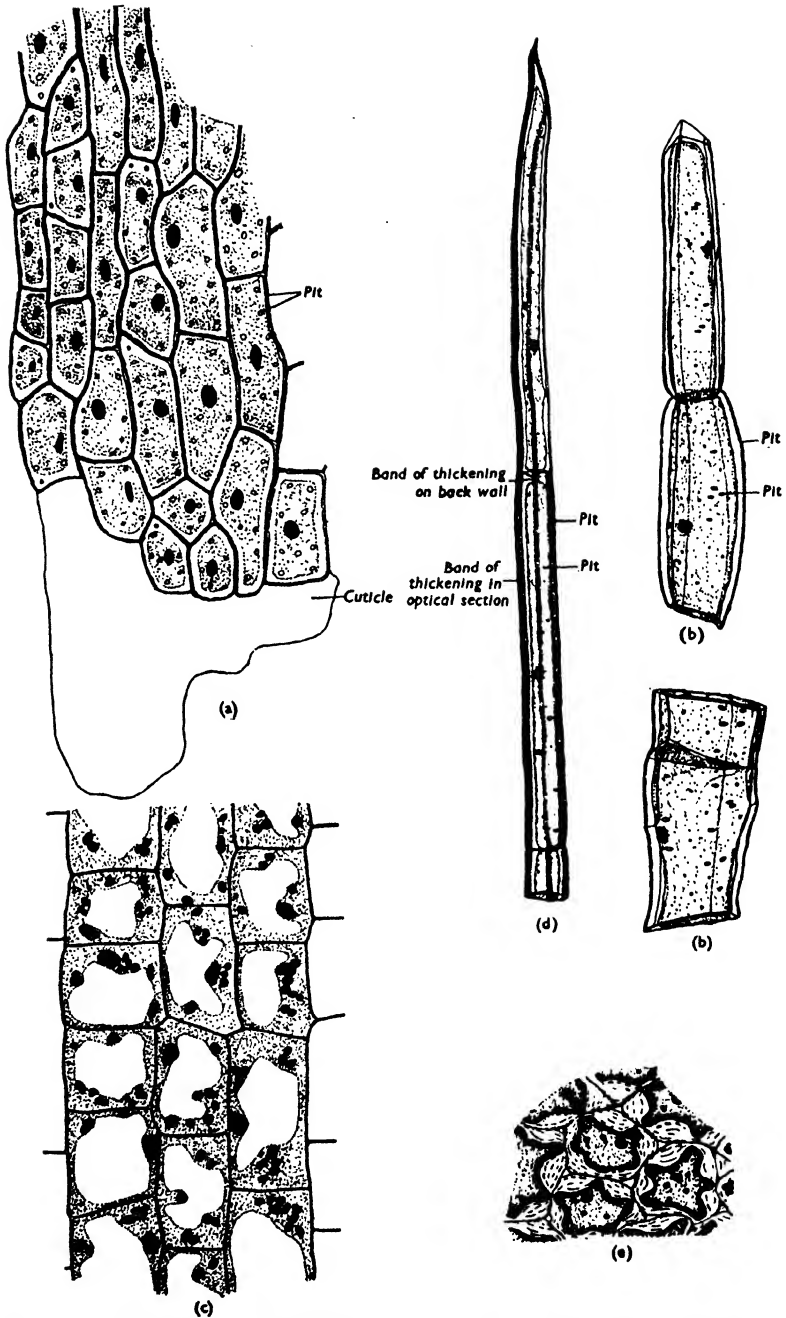


FIG. 72.—*Helianthus*. Elements from the macerated internode. (a) Epidermis seen from the inner side, at one end cuticle alone is left ($\times 200$), (b) cortical parenchyma ($\times 150$), (c) starch sheath ($\times 150$), (d) collenchyma ($\times 150$), (e) collenchyma in transverse section ($\times 250$).

The refractive appearance of these thickenings suggests that they are different in nature from the ordinary cellulose walls and that this is the case may be shown if a thin section is gently warmed in dilute potash, well washed in water, and then stained in dilute methylene blue. The walls of the collenchyma cells are much swollen by the potash and take up a violet colour with the methylene blue, which indicates the presence of pectin. In the macerated material the narrow collenchyma cells either remain in short files or become completely free. The characteristic thickenings of cellulose impregnated with pectin run as straight bars down the elongated cells, whilst the transverse walls remain thin and closely adpressed to one another. The bars only stain very faintly in hæmatoxylin; they may be seen in optical section on the side walls and, as the protoplasts contract away from the ends of the cells, they may be seen in surface view on the front or back walls as faint grey bands (Fig. 72*d*). These bars stain very well if the macerated material is mounted in dilute methylene blue. The presence of a large proportion of pectin renders the wall exceptionally elastic, so that peripheral cells of this type may undergo relatively large reversible changes in volume; the pectin compounds are also very retentive of water, a property that may be useful to the plant as these thickened walls border the intercellular spaces near the periphery of the stem.

When the walls of the epidermal cells in macerated material are examined under the high power, the radial and transverse walls, which are seen in optical section, have a beaded appearance due to the presence of numerous corresponding pits in these walls. Pits are also present in the walls of other cells of the cortex, where, however, they are less numerous. Pits in the walls of epidermis, collenchyma and parenchyma are illustrated in Fig. 72.

The Vascular Tissues, their Development in the Internode.

In the young stem immediately within the layer which gives rise to the starch sheath, lie the elongated, meristematic cells of the procambium, in close contact with one another and without intercellular spaces. Whilst epidermis and cortex have been differentiating, processes of differentiation in this

tissue have been giving rise to the vascular elements of the plant. In order to follow the early stages of development it is necessary to have a series of microtomed and stained preparations, cut transversely at successively lower levels through the young growing regions of a shoot apex. Any shoot will show the same stages, but the sections are more easily cut in a plant such as privet, with decussate phyllotaxis, as the shoot axis is straighter in such types. Staining with safranin and light green, or fast green, helps in the identification of some of the early stages. If such a series of transverse sections is followed

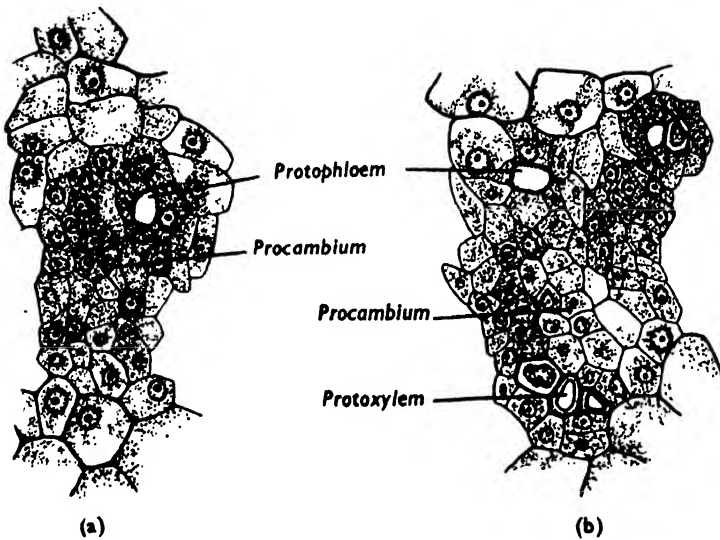


FIG. 73.—Transverse section of privet shoot apex ($\times 700$) to show (a) protophloem and procambium, (b) protophloem, procambium and protoxylem.

downwards, it is found that by the time that the level of insertion of the second pair of leaf primordia is reached, certain cells towards the periphery of the procambial tissue have begun to undergo changes. A cell, surrounded by meristematic cells, shows a swelling of the wall, which at the same time stains more deeply than its neighbours with the green stain (Fig. 73a); even in unstained material such swollen walls may be recognised by their more refractive, pearly appearance. Coincident with the wall thickening, the contents contract from the wall and gradually disorganise. Following the series down,

cells to the inside of the first to differentiate then pass through similar stages, whilst the older elements appear as empty, often as crushed, structures to the outside. They can be recognised for a time by the deeper green of the walls, but ultimately become almost impossible to identify. Cells undergoing this type of differentiation are losing their contents, and consequently fail to continue to grow, whilst the tissue around is extending in length, and so such cells are continually pulled out until they collapse completely. They are known as protophloem elements and, when once recognised near the apex of the shoot, similar cells can be followed uninterruptedly downwards, so that evidently protophloem elements differentiate upwards, in continuity with cells of the same kind below.

An individual protophloem cell is successively (*a*) a growing meristematic cell full of contents, (*b*) a protophloem element with swollen wall and disorganising contents, and (*c*) an empty, collapsing element. The contents lost from the cell as it differentiates and dies must obviously be available for the growing tissues around. Meristematic cells above and to the inside of such a differentiating cell, in their turn pass through the same stages. Thus by this very process of growth and differentiation, food is continually moving up the growing shoot towards the apical meristem and the young leaves. On passing down a series of shoot sections, the first appearance of protophloem is always in association with a leaf primordium, so that differentiation is earliest and most active in those regions of the procambial ring which form the leaf trace bundles.

The protophloem elements are transitory structures and also very small, so that their detailed structure is difficult to determine with certainty; it seems clear, however, that the end walls between vertically superposed elements become perforated and converted into sieve plates. The structure of sieve plates is better discussed in relation to the later formed and larger phloem, but the presence of perforated end walls suggests that the transfer of food through these elements is probably mainly in the longitudinal direction and so to the actively growing cells above.

After protophloem differentiation has commenced, the growth of the procambial tissue becomes very vigorous, and the cells grow and divide so actively that their increase can

no longer be accommodated by the extension in length of the internode. The transverse dimensions of the cells then increase, and this is followed by their division in the longitudinal direction, and especially in the tangential plane, so that a radial seriation of the cells in the middle region of the meristematic ring becomes apparent as seen in transverse section (Fig. 73*b*). It should be realised however, that at this stage growth in length and transverse divisions of the procambial cells are still proceeding as well, so that there would not be good alignment of the ends of the cells in a radial longitudinal section. The radial arrangement of the cells will naturally be most evident where growth in length of the organ is least and this is found to be the case in the region of insertion of a leaf primordium.

The second type of differentiation is that of protoxylem elements, which appear towards the inner side of the procambial ring ; occasionally they differentiate from irregularly arranged procambial cells, but more commonly from cells which show some degree of radial seriation in transverse view (Fig. 73*b*). The highest protoxylem element to be recognised makes its appearance at the level of insertion of and in association with a young leaf primordium. In this position, a single cell near the inside of the meristematic ring is seen to vacuolate before its neighbours, and its increase in size is rapidly followed by a thickening of the wall and a further disappearance of the cell contents. At the early stage at which this cell differentiates, internodes are not present, and this cell may extend from the level of one leaf insertion to that of the next leaf below. Around this cell and in contact with it, other cells vacuolate and enter upon a phase of vacuolating and dividing cell growth similar to that of the pith and cortex. As the walls of these vacuolating cells extend in length, the xylem element becomes passively drawn out whilst its protoplasm is decreasing in amount, apparently being used up in the deposition of secondary layers of cellulose upon its wall. The original primary wall of the cell is very extensible and readily stretches with the growth of the neighbouring files of cells, and the secondary layers of cellulose wall thickening are laid down upon this extending primary wall. Under these conditions of deposit, the secondary layers are always found distributed inside the primary wall, either as rings spaced at intervals along the inside of the tube, or as one or more

continuous spiral bands. The secondary thickenings gradually change in consistency, their staining properties alter, and the deposit becomes relatively rigid and inextensible. These properties, included under the term lignification, are much more easily recognised where they are common to larger areas of wall surface, and they are therefore discussed more fully when the later formed xylem is under consideration. Though they are relatively rigid, the annular thickenings may still be pulled farther apart, or a flat spiral may be pulled out into a longer, narrower one by the continued elongation of the surrounding tissues. As annular and spiral types of thickening do not prevent the continued extension of the element, they are often regarded as "adapted" for this purpose, but they are always found in xylem elements differentiated in extending tissues, and seem the natural result of a process of secondary wall deposition which has taken place under these circumstances.

If extension continues far enough, the thin primary wall between the secondary thickenings will probably collapse altogether, and the secondary wall thickenings will then form a series of disconnected, spiral or annular thickenings, as the surrounding parenchyma cells expand into the space originally occupied by the collapsed element (Fig. 71). Before this occurs, as the protoplasmic contents become too tenuous to retain the solutes in the sap, the liquid contents which were originally held in the turgid expanding element will have been released into the surrounding tissues. The first protoxylem element, which differentiates associated with the trace to a new leaf, is isolated from all previously differentiated xylem lower in the shoot. In its vacuolation it must have absorbed water, which was probably drawn from the older vascular system below, and when the sap is subsequently released from the differentiating element into the tissues around, these cells receive some of the sap necessary for their continued growth. So long as the leaf and the part of the stem below the leaf are growing and extending, a series of such xylem elements are being differentiated in the procambial strand common to both. As internode and leaf continue to extend, more xylem elements are differentiated farther out in the meristematic ring than the original element, and also others are

formed beneath and above those first formed, so that the xylem strand continues to differentiate radially outwards in the thickness of the internode and also upwards into the leaf and downwards into the extending internode.

When two elements, situated end to end, are differentiating in this way, their mutual expansion throws a great strain upon the thin primary walls that separate them. Very often these walls collapse under the strain and, when the protoplasts also disorganise, the cavities of a whole series of cells form one pipe, the vessel, the constituent cells of which are described as the vessel elements. In protoxylem vessels the primary walls are so thin that it is difficult to see whether the walls between the

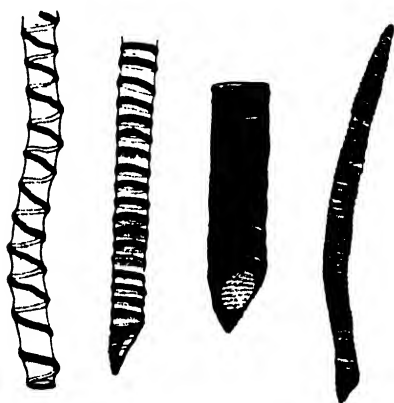


FIG. 74.—*Helianthus*. Xylem elements from macerated material of the petiole (two on left $\times 200$, two on right $\times 130$).

elements are perforated, but if material of sunflower is macerated in 5 per cent. chromic acid and then stained in Heidenhain's hæmatoxylin, the primary wall is stained faintly grey and the perforation at the end of the vessel element rendered visible (Fig. 74). In longitudinal sections which include a length of the same vessel, it is possible to demonstrate the continuity of the cavity if an air bubble is trapped in the

tube, when by gentle manipulation the bubble may be seen to move from one element to another through the perforations in the end walls.

In a transverse section of an extending internode of *Helianthus*, the first vascular elements appear as isolated elements or groups of elements in different patches of procambial meristem (the future vascular bundles), which lie all round the stem just within the starch sheath. In many dicotyledons the meristematic procambial elements in the young internode form a continuous ring, which is only broken at the node where parts of it are diverted as young vascular strands into the leaf. But in *Helianthus* numerous groups of cells, distributed

at intervals around this ring, have vacuolated at an early stage into parenchyma cells, which link the parenchyma of pith or medulla with that of the cortex. They are, therefore, often spoken of as medullary rays and, in view of their origin by vacuolation of cells of the procambial ring, they are termed *primary* medullary rays. They appear so early in development that the internodes are very short at the time, and the vacuolation of the original procambial cells and the continued growth of the parenchyma thus formed means the production of a ray extending throughout the length of several internodes. This great vertical length of the primary medullary rays distinguishes them sharply from the secondary rays, which are developed later in the vascular ring from cells cut off from the cambium.

The Distribution of Primary and Secondary Xylem in the Stem.

The protoxylem, as its name indicates, is the first xylem to differentiate; in the internode of *Helianthus* the elements may be recognised from later differentiated elements by their position near the inner margin of the procambial strand and by their smaller diameter, whilst in longitudinal view it is seen that the secondary wall deposits have an annular or loose spiral patterning and that evidently some elements of this type have been disorganised during tissue elongation. From this position xylem continues to differentiate in an outward direction from young cells of the procambium. In *Helianthus* this differentiation is proceeding whilst the internodes are extending, but the later the differentiation occurs in any particular internode the less the xylem elements will be pulled out by tissue extension, the wider their diameter and the closer the spiral patterning of the secondary wall. Finally as extension is ceasing, a few xylem initials will be pulled out to some extent, but will complete their differentiation after extension has ceased; the wall sculpturing of such vessel elements is found to be either reticulate, in which the turns of the spiral are connected in places so as to surround relatively large pits (Fig. 77*a*), or scalariform in which the secondary wall forms ladder-like bars of thickening between horizontally elongated pits on the wider facets of the elements (Fig. 77*b*). All this xylem tissue is

derived from procambial cells, or desmogen, and is described as primary; it is readily recognised, particularly in cross-section, by the fact that the only lignified cells are the tracheal elements (usually vessels) which lie amongst small-celled, unligified parenchyma (Fig. 75a). Primary xylem formation has been continuous, but the elements with wider lumen differentiated later than the protoxylem are called metaxylem; the demarcation between protoxylem and metaxylem is not sharp and merely indicates the direction of differentiation. In the leaf the procambium ceases growth when the leaf is fully expanded so that only primary xylem is present and of the vessel elements only the last differentiated will have reticulate or scalariform wall patternings.

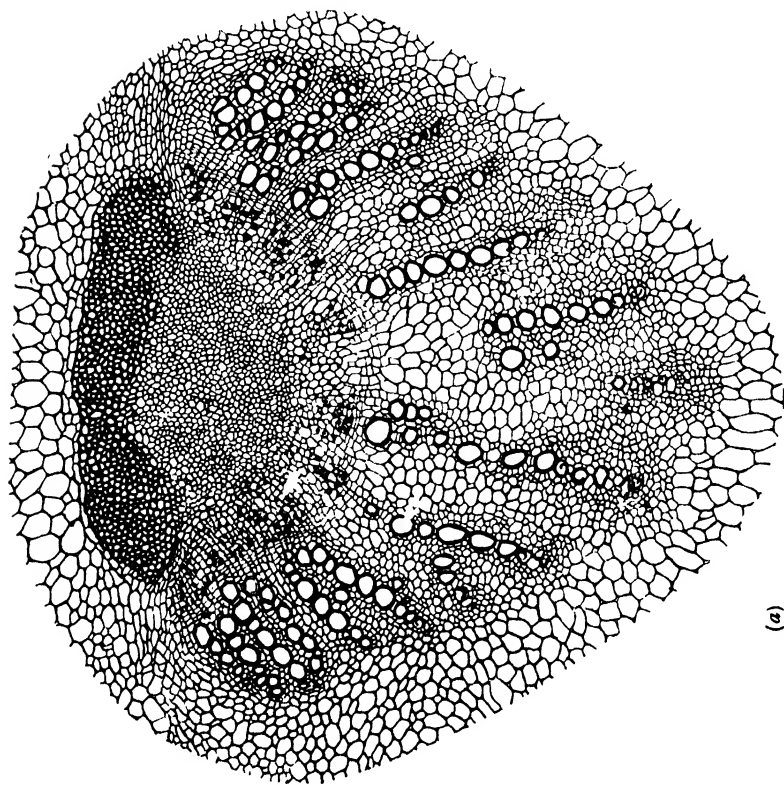
In the internode, however, the meristem in most of the bundles may continue, after extension of the internode has ceased, to divide as a cambium in which the divisions are orientated regularly in the tangential longitudinal plane. The cells formed towards the inner side by this activity continue to form secondary xylem outside the primary xylem, usually without any break between the two. In *Helianthus* the secondary xylem is recognised by the fact that all the elements are lignified (Fig. 75b); though the elements lignify almost simultaneously they are not all of one type, as is suggested by the difference in size of their cavities in transverse section and as will be clear when macerated material of secondary xylem is examined. In a perfect radial longitudinal section the cambial cells would show radial seriation and the ends of the secondary vessel elements would lie at approximately the same horizontal level as the ends of the cambial cells of the same radial series; however longitudinal sections are seldom likely to show this radial seriation over any appreciable distance owing to the difficulty of keeping the razor edge along the same line of cells.

In *Helianthus* the difference between primary and secondary xylem in transverse section, enables us to analyse a little further the types of vascular bundles which differentiate from the original groups of meristematic tissue within the starch sheath. Fig. 76 shows the outlines of the vascular bundles in an internode which has ceased to extend, and the typical distribution of primary and secondary xylem in them. To the outside of most of these vascular bundles there has now

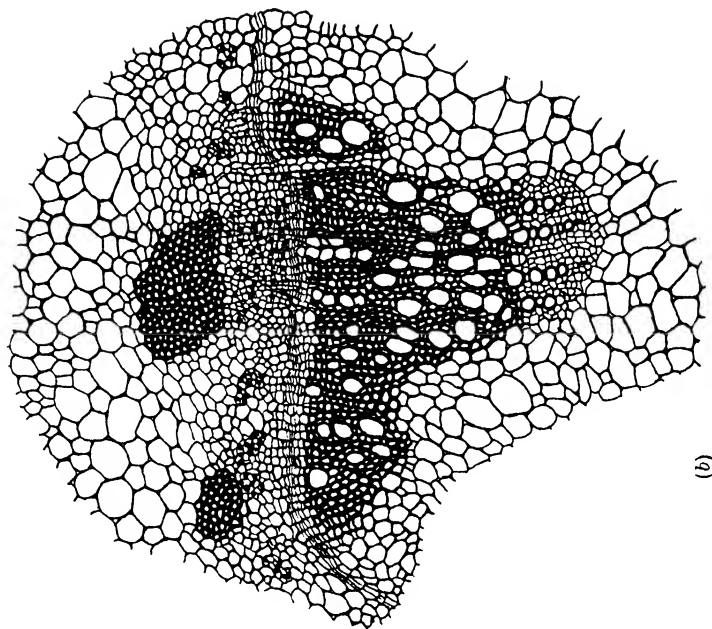
differentiated another tissue of cells with thick, lignified walls, the sclerenchyma.

In the following analysis it is necessary to remember that the internode examined is from the middle region of a sunflower stem, that a number of leaves have appeared on the stem above this level, and that the phyllotaxis in this region of a sunflower shoot is usually $2/5$ or $3/8$ (Chapter XIII).

Three prominent bundles, with slight ribs outside them on the surface of the stem, form the leaf trace (the whole bundle system entering one leaf) of the leaf inserted at the node immediately above. These leaf trace bundles have the maximum amount of primary xylem in them, because they were the earliest of all the bundles in the ring to differentiate in this internode, and therefore their differentiation has continued for a longer time during the period when the internode was extending. But these same bundles contain little secondary xylem, as, although the leaf above has now been adult for some time, since it reached the adult stage practically no more xylem has been added to the leaf trace, and, furthermore, the cambium on the face of the xylem of these trace bundles shows little sign of continued activity (Fig. 75*a*). The three leaf trace bundles of the leaf inserted at the node next above are also fairly prominent, and with experience it is possible to recognise the median trace bundles associated with still higher leaves (Fig. 76). In the trace bundles from higher leaves, the amount of primary xylem, at this level, diminishes and is replaced progressively by more and more secondary xylem. The disappearance of the primary xylem is readily understood. The xylem differentiation which starts at the level of a leaf insertion proceeds relatively rapidly and for a long distance down into the shoot; at first it differentiates down as primary xylem through internodes which are still extending, but then continues its further downward differentiation as secondary xylem through lower internodes which have already ceased to extend. It is important to realise that this appearance of secondary xylem in a fully extended internode only occurs at first in bundles which are in vertical continuity above with growing leaves; this is a most important conclusion for it shows the influence upon the vascular structure of the stem of the leaf system developed above it. The continued differentiation of secondary xylem is obviously associated with the meristematic



(a)



(b)

FIG. 75.—*Helianthus*. From the transverse section of an adult internode. (a) The median leaf trace bundle of the leaf of the node above ($\times 40$), (b) a synthetic bundle, \times of Fig. 76a ($\times 50$).

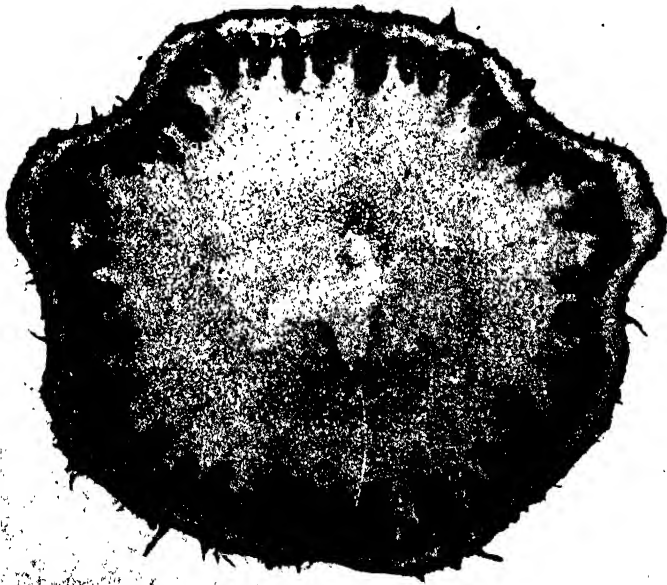
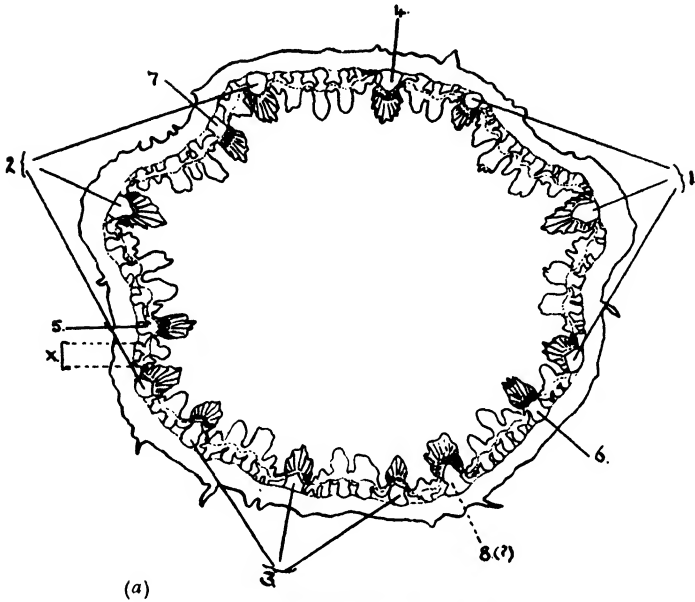


FIG. 76.—*Helianthus*. Photograph and plan of an adult internode in transverse section ($\times 4$). In (a) files of primary xylem indicated in the trace bundles.

condition of the cambium, but unlike the cambium in the median trace bundle of the next leaf above, the cambium on the face of the secondary xylem bundles, linked vertically with much higher and younger leaves and vascular elements differentiating in connection with them, is still vigorous and usually shows a tendency to spread tangentially. This effect is shown best by the "synthetic" bundles, formed when adjacent bundles from higher leaves become linked by common cambial activity (Fig. 75*b*).

In the synthetic bundles the primary xylem is absent or negligible in amount, but the secondary xylem produced by the active and spreading cambium soon gives them a wedge-shaped outline. It is the tangential spread of these bundles which is mainly responsible for the increase in the periphery of the vascular ring in *Helianthus* as the stem grows older; the vascular bundles are forced out on to a wider and wider periphery, with the result that the pith cells, which have ceased to grow, are unable to stand the strain and, after considerable passive radial elongation, are torn apart from one another, producing a widening pith cavity in the older internodes.

The continued radial growth and vascular differentiation of the internode is thus inseparably associated with the continued growth of the leafy shoot above this internode, a conclusion which proves of vital significance in the understanding of the radial growth of the tree. It is now necessary to consider the detailed structure of the elements composing the various tissues which have become conspicuous in the older internode.

CHAPTER XXIII.

STEM STRUCTURE IN THE HERBACEOUS DICOTYLEDON. II.

The Adult Internode, the Fibro-Vascular Tissues.

Within the starch sheath in the adult internode of *Helianthus* certain tissues have developed remarkably and, in particular, the xylem and sclerenchyma are conspicuous with their thick lignified walls (Fig. 75). The fibro-vascular tissues comprise elements of three different types. In the first category are the vascular elements, continuous between leaf and stem, present in every vascular bundle and which are regarded, for reasons subsequently to be discussed, as the channels of longitudinal transport of water and foods. These elements comprise the phloem and xylem, in which, as the internode grows older, secondary phloem and secondary xylem become more and more conspicuous. The secondary phloem in the older internodes has many points in common with the primary phloem, but the differentiated cells are of larger size and longer duration, since they are not pulled out by the longitudinal extension of the cells around; most of the primary phloem sieve tubes have by this time collapsed and this outer region has now lost the characteristic appearance of phloem. The primary xylem, owing to the rigid nature of the secondary wall deposits, still remains evident though less conspicuous than the more generally lignified region of the secondary xylem.

The region of the bundle between the starch sheath and the persistent phloem forms the lignified cap of sclerenchyma fibres; no conducting function has been attributed to this tissue. Sclerenchyma is not formed in all plants, but when present as in *Helianthus* it is so obviously closely associated with the vascular tissues that the whole bundle is described as a fibro-

vascular bundle. Between phloem and xylem will still persist the meristematic tissue, the cambium. These various tissues will now be examined in greater detail.

Secondary Xylem.

In a stained cross-section of *Helianthus* stem it will be evident that the secondary xylem comprises elements of different sizes, all of which have thick, lignified walls (Fig. 75*b*). The tissues we are dealing with now, when accumulated in bulk, form wood and these thick walls are said to have woody properties or to be lignified. Such walls have great rigidity ; they also have characteristic staining properties, such as a yellow colour with acid aniline chloride or sulphate, or a red colour after treatment with an alcoholic solution of phloroglucin, followed by hydrochloric acid. In the secondary xylem all the elements originally derived from the cambium become thick-walled and lignified. Only some of the elements, mainly those with the largest cavities in transverse section, become vessels, but they are embedded amongst other elements, which also have thick lignified walls. In the vessel elements the secondary wall deposits are laid down upon a wall which undergoes no further increase in length after its original expansion, which occurs while the wall is still thin and primary ; the secondary wall deposits are therefore laid uniformly over the whole wall, except for those regions originally occupied by groups of protoplasmic connections : these regions remain as thin places, the pits, in the otherwise thickened wall. Such an element is usually described as pitted (Fig. 77*c*), but in some cases the pits may be stretched into long, transverse ovals, when the thickenings between them resemble transverse bars and the element is distinguished as scalariform (Fig. 77*b*).

In typical xylem vessels and similar but unperforated elements known as tracheids, the thickened area of the wall increases towards the cavity of the element, so that the thickening overarches the pit, which thus forms a cavity in the thickness of the wall, with its widest expansion at the surface of the primary wall, which forms the pit membrane. Such pits are described as bordered. The cavity of the pit communicates with the cell cavity through the pit canal. The border is

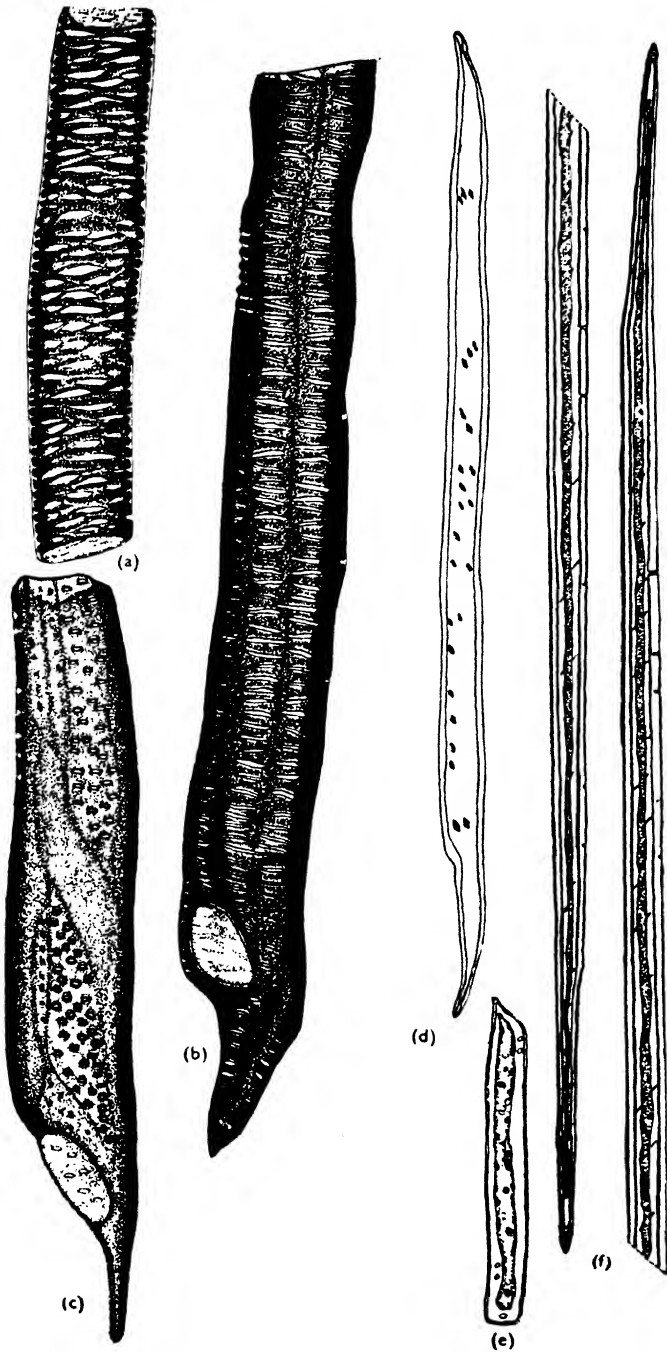


FIG. 77.—*Helianthus*. Tissue elements from a macerated stem (all $\times 240$).
 (a) Reticulate, (b) scalariform and (c) pitted vessel elements, (d) wood fibre,
 (e) wood parenchyma cell, (f) one sclerenchyma fibre in two lengths.

seen best in optical section of the wall ; in surface view the pit may be focused alternatively at the wider and fainter outline of the pit membrane, or at the narrower outline of the pit canal, which opens into the cell lumen (Fig. 78*a*). As the pits are left on the sites of the former protoplasmic connections, naturally they face one another upon opposite

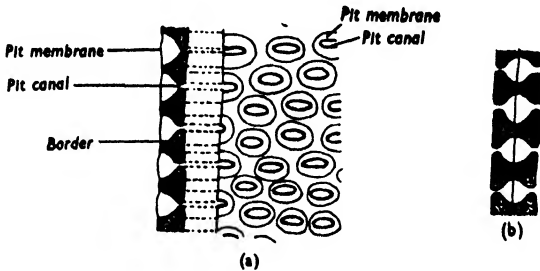


FIG. 78.—(a) Diagrams of bordered pits of a vessel element of *Helianthus* in surface view and, to the left, in optical section, (b) adjacent walls in optical section to show correspondence of pits ($\times 750$).

sides of the wall so that pits upon adjacent elements normally correspond (Fig. 78*b*)¹.

When the vessel elements of the secondary xylem increase in volume during differentiation, and the thin cross walls are stretched beyond their capacity for extension and perforate, the remains of the perforated membranes contract to form the narrow rims of the adjacent elements. If the end walls of the vessel elements are oblique, the strain upon them due to the expansion of the cell is less severe, and such cells may develop only a small perforation with a wider rim or may even fail to perforate altogether. When the expanded wood element, with its characteristic, bordered pitting on the wall, is not perforated at either end, it is known as a tracheid (Fig. 74), and both tracheid and vessel may be classed together as water-conducting elements. In addition to vessels, the secondary xylem contains wood parenchyma cells, which retain their living contents, and elongated, thick-walled cells which lose their contents during development and form the wood fibres. Elements of these two kinds may be identified in macerated xylem from the stem of *Helianthus* (Fig. 77*d* and *e*), but they are both

¹ The pit in the wall of a single element may be called a half pit, the corresponding half pits forming the pit.

seen more clearly in sections and macerated material of a more woody stem, so that their description in detail is deferred to the next chapter.

Metaphloem and Secondary Phloem.

In a transverse section of *Helianthus*, the phloem, which lies between the cambium and the thick-walled cap of sclerenchyma, is readily recognised on account of the unglified walls of the cells and the characteristic types of element. Many of the cells are vacuolated and relatively wide in cross-section, but each of these is associated with a small cell which remains densely packed with protoplasm (Fig. 71). Each large cell is an element of a sieve tube, whilst the small cell, cut off from the mother cell of the sieve tube element at an early stage of development, is known as a companion cell. In the section some of the sieve tubes may appear to be empty, others have contents which are probably mucilaginous in nature and stain deeply, whilst in some the relatively transverse end walls may be seen in surface view and these usually appear to be covered with fine dots. Associated with these elements are some vacuolated elements of intermediate size which are phloem parenchyma cells. The phloem as a whole is a narrow celled tissue which is not easy to examine in the longitudinal view; the best method is to use material macerated in chromic acid and stained in Heidenhain's hæmatoxylin. In this material it is possible to identify the long narrow sieve tube elements, often still attached end to end as in the sieve tube. Still attached to these may often be seen the dense nucleated cells which commonly arise by transverse divisions of the companion cells; a file of such cells will correspond in length with the element of the tube (Fig. 79). In the material there will also be present some long narrow cells with less dense contents, the phloem parenchyma. In the sieve tube element itself the nucleus disappears early and the protoplasmic contents seem to break down into a mucilaginous slime which stains deeply. Whilst this remains in close contact with the transverse wall, it is often contracted away from the longitudinal walls and appears as a dark strand up

the centre of the tube and usually denser at one end of the element than at the other (Fig. 79a). The sieve tubes are so narrow in *Helianthus* that the details of the end walls of the elements are difficult to make out, but in optical section there may be some indication that they are perforated by numerous holes, through which the slimy contents are continuous; in surface view, when the contents are drawn away from the end wall, the latter is seen as a very fine network. On this account the contents of the sieve tube elements are regarded as continuous through the plates, and the elements thus constitute a long tube in which the original protoplasmic contents of the constituent cells are much disorganised and ultimately disappear. Indeed, secondary, like primary phloem, gradually loses its contents and the elements become empty, soft-walled tubes, which tend to collapse except where they are still expanded by the presence of transverse walls.

In most herbaceous stems, as in *Helianthus*, the phloem consists of narrow elements of which the detailed structure is difficult to determine. Therefore this tissue is usually examined in *Cucurbita*, where the phloem is exceptionally well developed. In transverse section of the stem the vascular bundles of the plant are seen to be arranged in two rings, and the five smaller bundles, which form the outer ring, lie opposite to the five longitudinal ridges on the stem. No typical sclerenchyma is developed, but a ring of thick-walled lignified tissue lies near the periphery of the stem.

In *Helianthus* the bundles are of the typical, collateral type with xylem, cambium, and phloem arranged in radial series, but in *Cucurbita* the bundles are bicollateral, since they have an additional group of phloem elements internal to the xylem. Normal cambium is present between the xylem and the outer phloem; between the xylem and the inner phloem it usually ceases activity early and gives rise only to phloem. In any of the patches of phloem the sieve tubes are seen to be very much larger than in *Helianthus*, though the same characteristic features are present. Most of the sieve tubes are cut across between the sieve plates so that the large, almost empty cavity of the tube is seen surrounded by the thin wall, and associated with one small, dense companion cell (Fig. 80), or sometimes two similar cells. Rather nearer the xylem younger elements may still be present which still show a

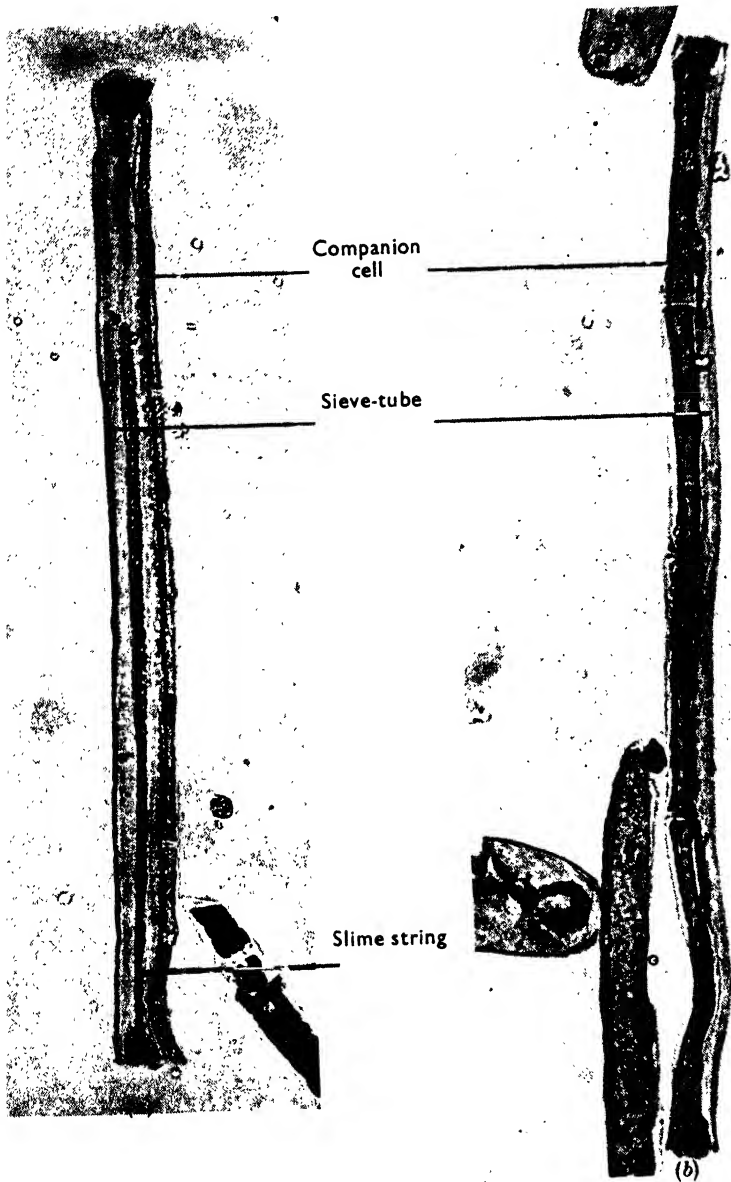


FIG. 79.—*Helianthus*. Photographs of sieve tube elements from macerated phloem. A file of companion cells lies to the right of the element in (a), and over the element in (b) ($\times 400$).

contracted protoplast, and this may be granular with contained starch grains or more homogeneous if further disorganised. Other tubes may be cut so as to show the transverse

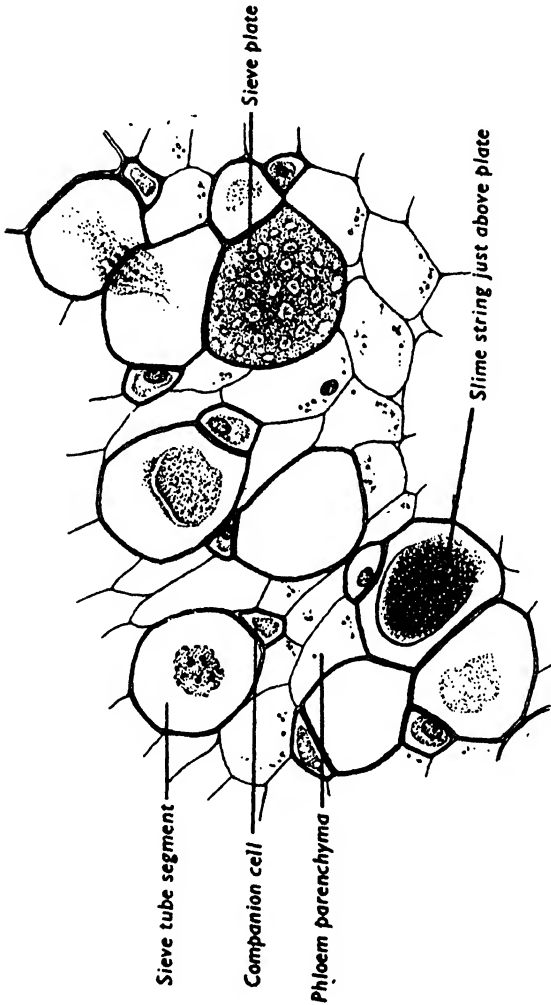


FIG. 80.—*Cucurbita*. Part of the phloem from an internode in transverse section ($\times 400$).

sieve plate in surface view, when the relatively large perforations are clearly seen. In permanent preparations stained with safranin and light green, the younger cross walls stain

green like the cellulose walls of the parenchyma, whilst some of the others stain red with the safranin. The red staining is due to the accumulation of a curious thick deposit, which is apparently derived from the disorganising protoplasm; it gradually encroaches on the slime strands which pass through the perforations of the plate and may eventually block up the pores altogether.

This substance, known as callus, appears to be a carbohydrate and has characteristic staining properties, of which one of the more distinctive is its affinity for aniline blue. Both callus and the mucilaginous slime strings attached to it are very resistant to concentrated sulphuric acid, so that if longitudinal sections through the phloem are mounted in concentrated acid, the cellulose walls rapidly dissolve, leaving the callus pads of the old sieve tubes still intact, with thin slime strings attached to them. Outside the expanded sieve tubes a line of crushed cells indicates the position of the protophloem, which has been completely crushed by the continued growth of the stem.

In macerated and stained material sieve tubes of all ages may be examined. It is usually possible to find some sieve tube elements still attached to one another at the sieve plates, and in such cases it may even be

possible to see the continuity of the dark stained slime strings through the pores. The younger tubes are recognised by the presence of starch grains in the contracted protoplast, whilst the older ones may have a central slime strand, which stains deeply and expands to cover the area of the cross wall, where callus may also be forming over the perforated plate.

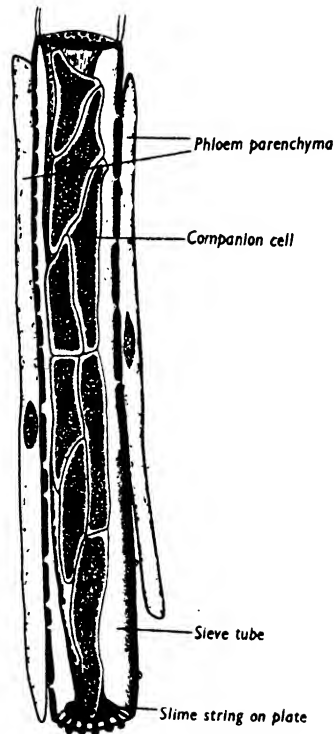


FIG. 81.—*Cucurbita*. An element of a sieve tube; pits are seen in optical section in the walls making contact with parenchyma cells, companion cells lie over the element ($\times 300$).

Fig. 81 shows the types of element that occur in contact with sieve tubes. These may be long vacuolated parenchyma cells, which may be approximately the same length as the sieve tube element or shorter. The original dense companion cell undergoes transverse divisions as in *Helianthus*, and frequently some longitudinal divisions also. In some places two sieve tubes may be in contact laterally, and in this case oblique or vertical sieve plates occasionally occur between them. In the stained material the longitudinal sieve tube walls also show characteristic thin areas. In the transverse walls which develop into sieve plates, the wall also had originally thin areas penetrated by groups of protoplasmic connections, but during the process of expansion these thin areas become completely perforated, so that only the network of thickened wall remains as the plate. In the longitudinal walls the thin regions persist, so that these are really equivalent to pits. The photograph of the isolated sieve tube element in Fig. 82a shows the perforated sieve plate at one end, the numerous transversely extended pitted areas on one facet of the tube which was in contact with companion cells, and two facets with more rounded pits which were in contact with parenchyma cells. In Figs. 81 and 82b the rounded pits are seen in optical section.

Sclerenchyma.

A feature which is very variable from one plant to another is the extent to which the thick-walled tissue known as sclerenchyma is developed. In *Helianthus* it is strongly developed and lies external to the persistent phloem. Since it is developed internal to the starch sheath and shows no radial seriation, it has arisen from the elongated meristematic cells of the procambium (Figs. 71 and 75), but we have seen previously that this is also the region in which the protophloem sieve tubes develop, so that evidently sclerenchyma in *Helianthus* is a later development of the residual cells of the protophloem region. In plants like *Cucurbita* which form no sclerenchyma the whole protophloem region is crushed by later stem expansion and is recognised in older bundles as an arc of crushed cells outside the persistent phloem, whilst in still other plants sclerenchyma-like fibres may be developed outside the vascular bundle.

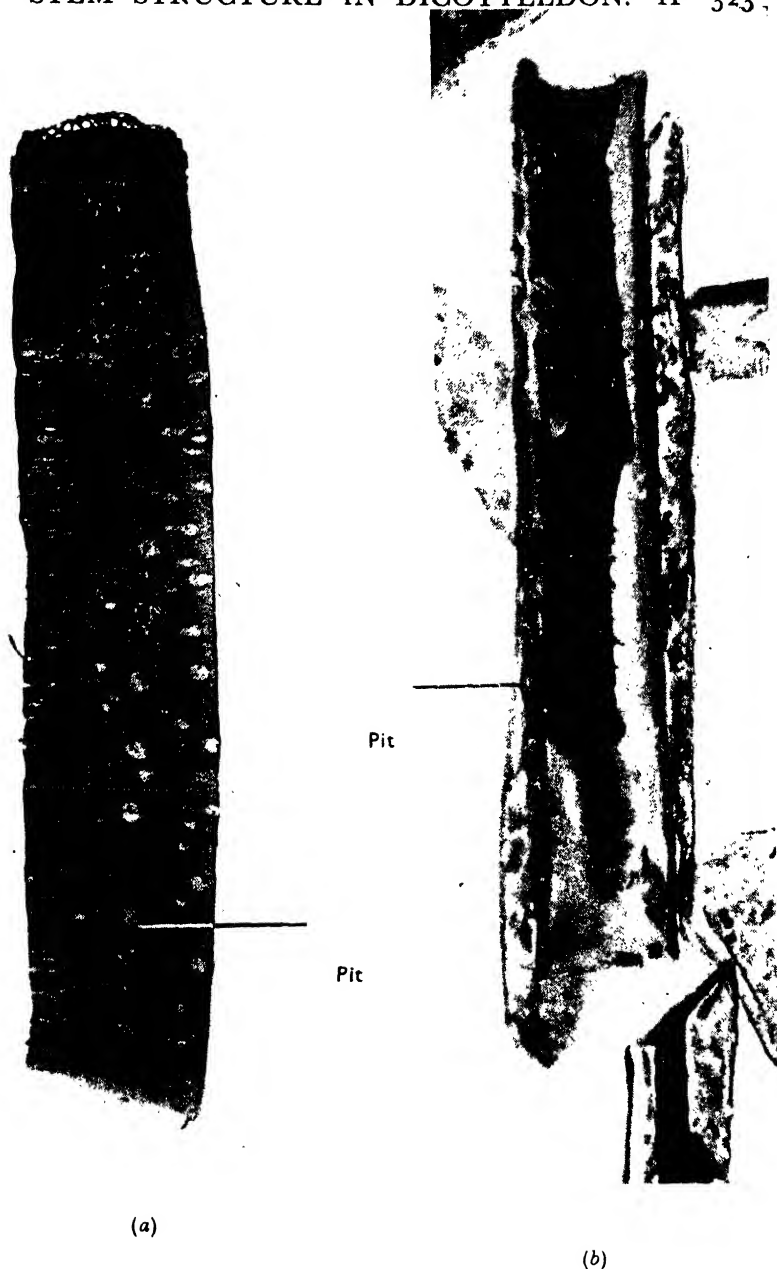


FIG. 82.—*Cucurbita*. Sieve tube elements from macerated phloem ($\times 425$).
(a) Showing pits on the longitudinal walls in surface view,
(b) in optical section.

In plants which form sclerenchyma in the protophloem region, the meristematic cells continue to grow in length for a long time. They may commence their growth in the axis at such an early stage that node and internode are not yet differentiated, and if they continue to grow without transverse division, they may finally extend through several internodes. In many plants they may reach a length of several millimetres and in the case of ramie (*Boehmeria nivea* Gaudich) they reach the amazing length of 40 to 50 centimetres.

When such cells extend through several internodes, they are in the curious position that the lower end of the same cell may be in a tissue which has ceased to grow in length, whilst the upper end still lies in a zone of extension. Unlike collenchyma, wall thickening never takes place in this tissue until growth in length has ceased, and in ramie sclerenchyma cells have been seen in macerated material which have commenced to thicken their walls at the lower end, whilst still growing in length by expansion of the primary wall at the upper end. In this case the process of wall thickening consists in the deposition of successive cellulose lamellae, and these begin to form at the lower end and gradually work up the inner surface of the cell as a series of hollow cylinders, which are finally completed to the upper end of the cell when this ceases to extend. In macerated material of *Helianthus* stem, the individual sclerenchyma fibres are seen as elongated fibres, much longer than the wood fibres, usually with somewhat pointed ends. With increasing age the walls become very thick and lignified, though this does not take place until late in this tissue, and in many plants no lignification occurs at all. The walls have a tendency to be tough rather than brittle and the mechanical properties of the fibres, in which the cellulose is constructed of carbon chains arranged in a very steep spiral, almost parallel to the long axis of the cell, renders them very resistant to pulling strains and therefore ideal materials to spin into strong threads. Thus linen thread is made from such fibres in flax (*Linum usitatissimum* L.) and other threads from fibres in hemp (*Cannabis sativa* L.) and jute (*Corchorus* spp.), and, apart from cotton, most vegetable fibres used in textile industries are sclerenchyma fibres.

From the base of the stem of periwinkle (*Vinca minor* L.) these fibres can be obtained for examination in a very simple way. When the basal region of the stem is roughly broken across, the fibres project from the broken surface as fine silky threads, which can easily be drawn out with forceps and mounted for examination. Though unlignified and therefore unstained in aniline chloride, this reagent brings out the spiral structure of the wall, which in this plant is especially interesting, as, in a single thickness of wall, two distinct spirals are present which cross each other, always at a very constant angle. This is seen more clearly after gentle compression of the fibres.

In *Vinca*, probably owing to the rather coarse texture of the wall, the pits are difficult to see, but in a yellow loosestrife of the gardens (*Lysimachia vulgaris* L.), both in sections and in macerated material, the pits are readily visible, and in optical section are seen as long canals in the thick wall, in surface view as narrow slits. As in the case of the small round pits of parenchyma, the opening of the pit to the cell cavity has the same dimensions as the pit closing membrane, so that the side of the pit cavity is straight; such pits are described as simple, as compared with the bordered pits already described for vessels.

The Cambium.

In the bundles in the transverse section of an adult internode of *Helianthus* the radially compressed, thin-walled cells of the cambium are recognised between the xylem and the phloem, and the same radial seriation can be followed into the tissues of the xylem and phloem (Fig. 75*b*). In a relatively young internode the bundles are separated from one another by parenchyma of the primary rays, which is continuous from pith to cortex, but as the stem grows older, the characteristic cambial type of cell division, by tangential longitudinal walls, spreads out from the bundles, and first from those with the most secondary xylem, across the intervening parenchyma (Fig. 75*b*) until the cambium eventually forms a continuous ring all round the base of an old stem.

Although the arrangement of tissue described for *Helianthus* is general for herbaceous dicotyledon stems, various

modifications of it occur in different plants, as for example the bicollateral type of bundle seen in *Cucurbita*. In some other stems, such as *Phlox*, the leaf trace bundles are not so conspicuous, as the protoxylem of the leaf trace bundles is scattered more evenly around the original procambial ring. In *Phlox*, *Lysimachia* and other plants with an unbroken vascular ring, radial seriation of the cells is seen all round the vascular ring after cambial activity has commenced.

CHAPTER XXIV.

THE WOODY STEM. HARDWOOD AND SOFTWOOD.

IN the herbaceous shoot procambial growth of a trace bundle in the internode is associated with the growth of the leaf inserted at the node above, but when that leaf is adult or has fallen the internode may continue to grow in girth by the activity of a cambium so long as there are still growing leaves at higher levels on the same stem. This means that the lowest internodes on such an herbaceous stem continue to increase in girth so long as the shoot apex continues to grow. Most of the new tissue formed by the cambium is cut off to the inside of the meristemetic cambial cylinder and differentiates into wood, so that the basal internodes become progressively more woody as growth in girth proceeds. Thus in herbaceous stems the basal internodes may become distinctly woody, but the process is very much more marked in the stems of woody perennials in which, at the end of one seasons growth, the stem is so woody that it is able to persist over the winter, and the next year growth is resumed by the extension of shoots from the buds left at various nodes on the stem. In the new shoots cambial activity is initiated in the same way, but the essential characters of the woody plant are due to the fact that this cambial activity does not stop at the base of the new shoot, but is continued right down over the surface of the wood of the shoots which underwent extension growth in earlier years. This results in the addition of a radial increment of wood over the surface of the old wood down as far as the base of the tree, and gradually there is built up a massive system of woody branches, of which the structural features are almost entirely due to secondary tissues derived from the activities of the cambium. These will be examined in a very preliminary way in this chapter and, whilst

our account will be based mainly upon the behaviour of the woody dicotyledons, some reference will also be made to that other characteristic group of woody perennials, the conifers.

The forester and the timber merchant derive their timbers from trees of both these groups and describe them, from their characteristic wood-working properties, as hardwoods (dicotyledons) and softwoods (conifers). We will therefore extend our examination of these woody structures far enough to enable us to recognise the most characteristic structural differences between these two types.

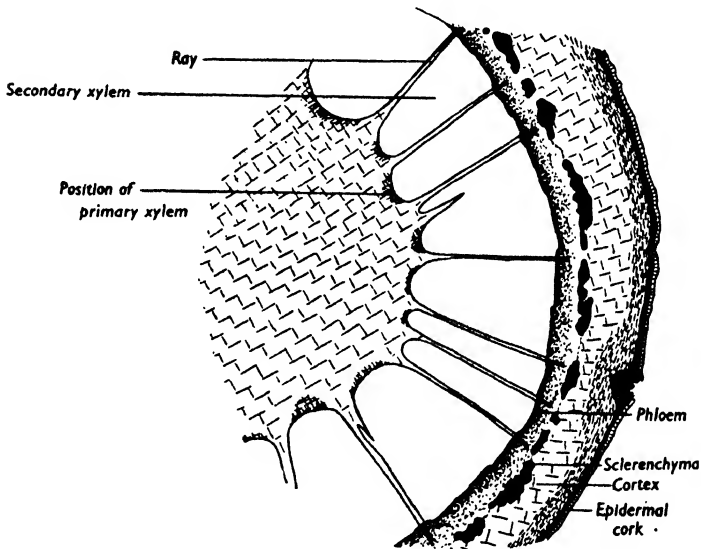


FIG. 83.—Apple. Low power plan of a transverse section of an internode of a one-year old stem ($\times 30$).

The Dicotyledon Woody Shoot in the First Year.

In order to recognise the main structural differences between a herbaceous and a woody stem, it is convenient to take a transverse section of a one-year shoot of apple (*Malus pumila*). This may be stained in iodine, followed by aniline chloride and finally mounted in glycerine. The ring of leaf trace bundles, which are often conspicuous in the herbaceous stem, is seen to have been replaced by a broad zone of lignified wood, outside which is a narrower ring of phloem with peripheral groups of thick-walled lignified sclerenchyma. The

primary wood of the original bundles projects into the larger-celled pith as radial rows of vessels surrounded by parenchyma. The pith in apple and in many other woody stems is lignified, though otherwise similar to normal parenchyma. From the groups of primary xylem the much more conspicuous secondary xylem radiates out into wider blocks which are only separated from one another by narrow rays of radially elongated parenchyma, one or two cells in width (Fig. 83).

Secondary phloem showing radial seriation occurs in a zone outside the cambium, but the position of the protophloem of the original leaf-trace bundles can only be located by the patches of lignified sclerenchyma which have developed from the residual procambial cells of this region and which form peripheral caps to the primary phloem as in *Helianthus*. Outside the sclerenchyma lie the parenchyma and collenchyma of the cortex and the epidermis. The latter may appear to be separated from the collenchyma by a few cells in radial alignment, this is the first indication of cork formation.

The secondary xylem is the most characteristic feature of the woody stem. All that we

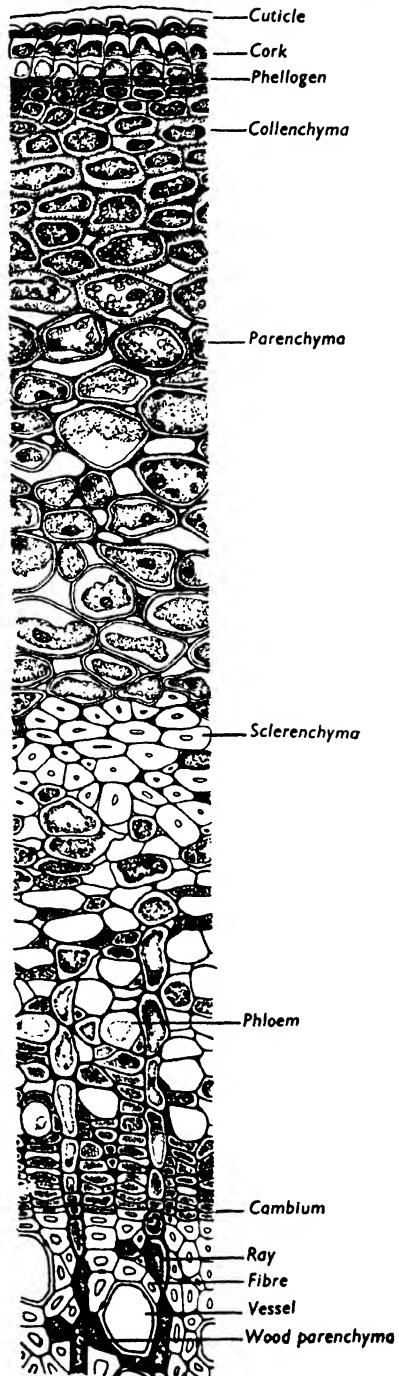


FIG. 84.—Apple. A sector of the internode, shown in Fig. 83, under high power ($\times 360$).

can note in the transverse section is the dispersion through the wood of relatively wider vessels with lignified walls amongst other elements which have narrower cavities but equally thick, or even thicker, lignified walls. In the iodine stained section the parenchyma cells of the rays are seen to have contents which include starch in autumn, and similar starch-containing, though not radially elongated, cells appear scattered in the wood between the rays and are known as wood parenchyma. The remaining narrow and empty elements are wood fibres (Fig. 84).

In the ring of secondary xylem the rays are conspicuous because they are the only radially extended elements, and also because they continue their radial course through wood, cambium and phloem. If the radial rows are examined in the region of the cambium, it is seen that, whereas the cambial cells on the face of the normal xylem are very flattened in the radial direction and have thin tangential walls, on the face of the rays the cells of the cambial ring are much less flattened (Fig. 84). In fact it seems clear that the ray initials are vacuolating and dividing cells in the ring of narrow, flattened elements of the cambium which behave like eu-meristem. In longitudinal sections it is seen that the ray initials are not elongated in the longitudinal direction, and they obviously resist the radial compression to which the elongated, meristematic cambial initials readily yield. The vacuolating ray cells do not undergo tangential longitudinal divisions so frequently as the cambial cells, but as they have a greater radial extension they keep pace in growth with the rest of the periphery of the wood and the cambial ring spreads outward as an unbroken ring, although it is actually a ring composed of two types of cell. The radial arrangement of the secondary phloem elements shows that this tissue also is increased by cells cut off by tangential divisions from the cambium, and on this side also the ray initials are adding new cells to the ray. On the outside of the secondary phloem, just within the thick-walled patches of sclerenchyma, the older phloem tissues appear very empty and partly collapsed, so that it becomes impossible to recognise the primary phloem groups with their lack of radial order.

Since this internode completed its elongation growth, the amount of secondary xylem formed has obviously been very

considerable, and during its formation it must have pushed the original cortical and epidermal tissues farther and farther outwards, at a time when these tissues were fully vacuolated. The result is that the cells of the epidermal and cortical tissues became extended in the tangential direction and some radial divisions occur. In addition the epidermis becomes replaced by a thin zone of radially arranged cells, which result from a new type of activity in which cell divisions have been taking place almost entirely in the tangential plane. That the divisions have originated in the epidermis itself is shown by the fact that the radial series may be followed right to the periphery, where the outermost segment is obviously the outer part of the original epidermal cell with its outer covering of cuticle (Fig. 84). The outer layers of new cells soon lose their contents, their walls become thick and dark-coloured and are evidently fat-impregnated, since they stain with Sudan III in glycerine after warming, like the original cuticle. These are the cells of the cork, a new sheet of cells with fat-impregnated walls beneath the cracking and distended cuticle. To the inside of the cork cells is a layer of radially flattened living cells on the same radial series as the cork; in these cells of the cork cambium or phellogen tangential divisions may still occur. Later we will return to consider the changes in the superficial tissues of the woody stem which are initiated by this replacement of cuticle by cork, but we must first study in more detail the changes in the vascular tissues, and particularly in the woody cylinder, since this is the most striking feature of the prolonged radial growth of the woody stem.

The Structure of the Wood.

The most suitable material for a study of the wood is provided by a transverse section, in conjunction with longitudinal sections, and material of the wood macerated in 5 per cent. chromic acid and stained in Heidenhain's hæmatoxylin. When the macerated material of apple wood is examined, the commonest type of element is the wood fibre. The fibres are long elements with pointed ends and the thickness of the wall is equal to about half the width of the cell lumen. The bordered pits on the fibres are characteristic, and appear in surface view as light slits which run obliquely in the otherwise blue-grey stained wall

(Fig. 85*a*). The pits on the upper wall of the fibre all slope in the same direction and have the same pitch, but at a lower focus similar slit-like pits are seen on the back wall which slope at the same angle to the longitudinal axis of the cell, but in the opposite direction to those seen on the upper wall. It is obvious,

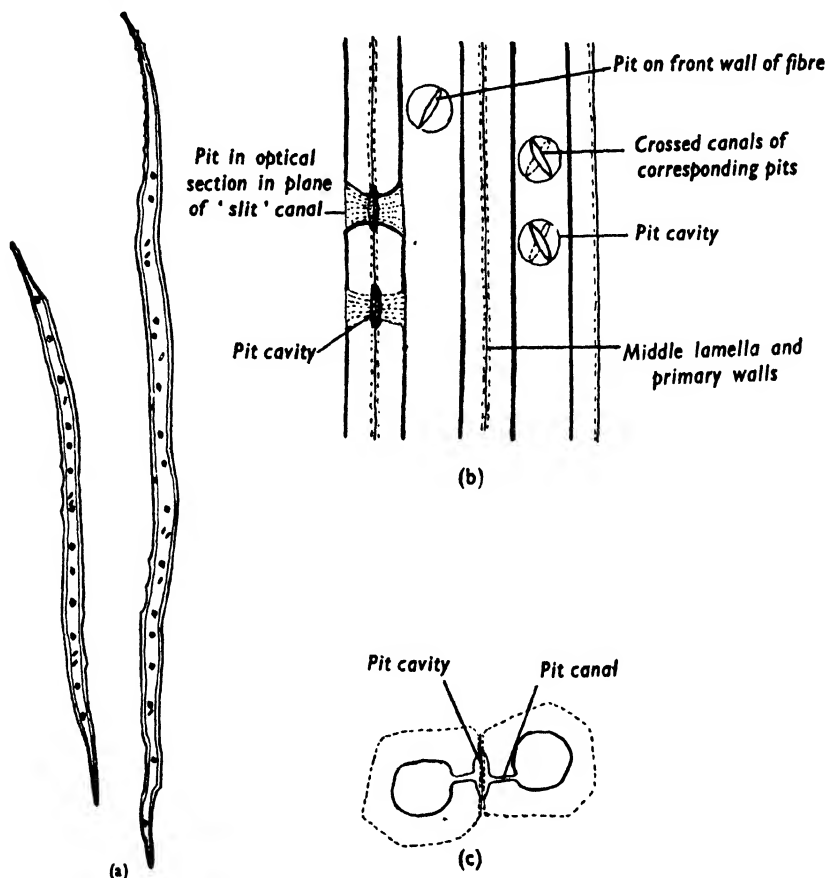


FIG. 85.—Apple. (a) Fibres from macerated wood ($\times 100$), (b) surface view and optical section of corresponding pits in fibres in a longitudinal section of the wood ($\times 800$), (c) corresponding pits between two fibres in a transverse section of the wood ($\times 800$).

however, that by this method of inspection, we are looking at the upper wall from the outside, and the lower wall from the inside, and this is the reason for the apparently opposite direction of slope of the pits, which actually all lie in the pitch of the same spiral round the wall. In some of the isolated fibres it may

be possible to see a pit in optical section by focusing on the longitudinal wall, but this view is usually seen better in longitudinal sections. In this view the pit canal, which is the conspicuous part of the pit in surface view, is seen as a slit; it runs from the cell lumen through the greater part of the secondary layers of wall thickening. As the section is nearly in the plane of the slit, this appears wide in this view (Fig. 85*b*). Away from the cell lumen the pit canal opens into a wider cavity, where the original pit region of the primary wall has been encroached upon by the secondary wall. In apple fibres the cavity of the pit is almost circular and shallow in comparison with the pit canal which is slit-like, with a slit about equal in length to the diameter of the pit cavity. The direction of the spiral on which the slit-like pits lie is constant for different fibres in the same plant. Consequently when the pits are seen in surface view in fibres in a longitudinal section, where two adjacent fibres may be thought of as lying back to back, the slits of the two pits appear to cross (Fig. 85*b*). These canals open into the cavities of the two corresponding pits in the adjacent fibres, which are separated from one another in the region of the pit by the middle lamella and the primary walls of the two elements, which together form the pit membrane. In transverse sections the deep pit canal, narrow in this view, and the shallow cavities of the corresponding bordered pits between adjacent fibres, are readily seen (Fig. 85*c*), and this is probably the best view from which to gauge the relative depth of the canal and cavity.

The vessel elements are readily distinguished from the fibres as they are wider by about three to six times and only about one-half to one-third the length. At each end there is a perforation bordered by a thicker rim, which is all that remains of the original cross walls between cells which have become the elements of a vessel. To one side of this rim, in apple, the element tapers to a point and in practically no case is the perforation truly horizontal. As seen from the transverse section, a vessel may lie next to any of the other types of element which compose the wood, i.e. fibres, other vessels, wood parenchyma, or ray parenchyma, and when a vessel element is seen isolated, the type and arrangement of the pits on it give an indication of the contacts made by that

particular vessel element in the wood (Fig. 86*a*). The surface facing upon a fibre is often not pitted at all and thus appears as a vertical, evenly stained strip. Where the vessel has been in contact with another vessel, the pits, which are not very crowded, have an oval pit canal surrounded by a faint border.

The long axis of the oval pit canal is horizontal in the wider shorter vessel elements and slightly oblique in the longer narrower ones. Facing ray parenchyma the vessel wall develops square or transversely extended rectangular areas with closely crowded pits, in which the border is very narrow, the pits themselves are oval or almost circular. Facing wood parenchyma the pits

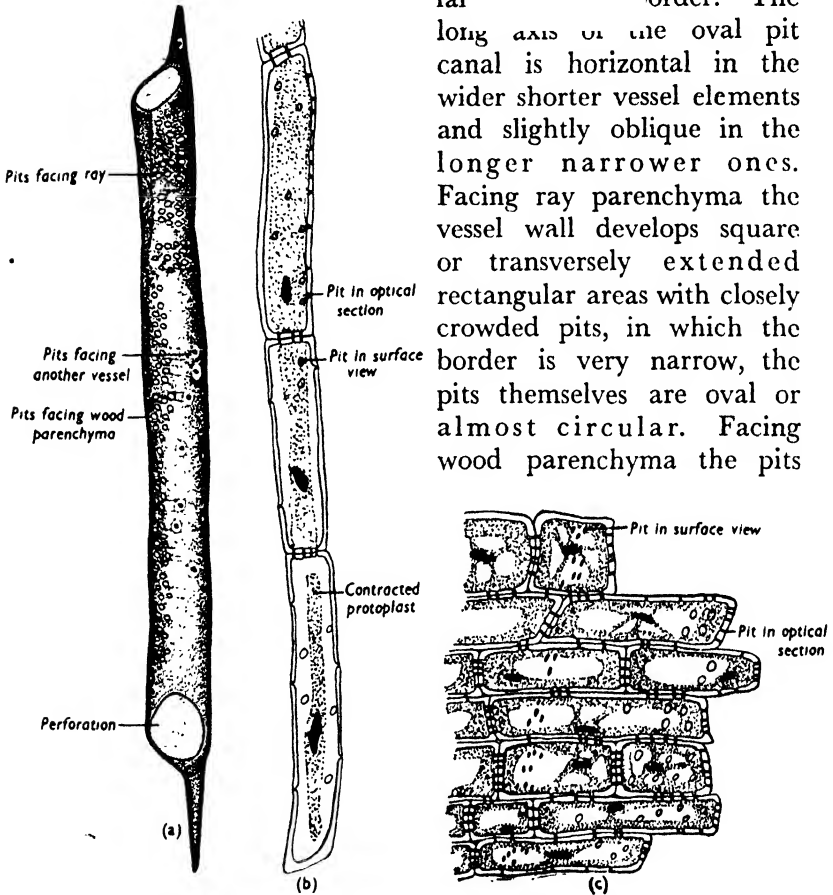


FIG. 86.—Apple. Elements from macerated wood. (a) Vessel element ($\times 270$), (b) wood parenchyma ($\times 400$), (c) ray parenchyma ($\times 400$).

are similar to those formed in contact with ray parenchyma, but the pitted areas are narrow and elongated in the same direction as the long axis of the vessel.

The wood parenchyma cells are narrow and elongated and occur in short vertical files (Fig. 86*b*), of which the middle cells are square-ended, whilst the distal cells are pointed at

the end which terminates the file. The pits are numerous and described as simple as the pore in the wall has a straight margin, so that no border is seen. These cells are often seen still in contact with a vessel element. The ray cells are relatively shorter and wider than the wood parenchyma cells, and are often found in the macerated material still adhering to one another in short horizontal bands (Fig. 86c), in which the longer axis of the cells would have been actually at right angles to the long axis of the other elements in the wood.

When elements of different kinds are in contact with one another, each one tends to keep its characteristic type of pitting, though the adjacent element may modify the pitting to some extent. Thus where a parenchyma cell is in contact with a vessel, the corresponding pit is simple and unbordered in the wall of the parenchyma cell and bordered on the side of the vessel, though the border is narrower than in the case of pits between adjacent vessel elements.

In the wood of some trees, e.g. oak (*Quercus* spp.), tracheids are present in addition to the elements described in apple. In this wood many of the vessels are very wide and the tracheids occur mainly around the vessels. The tracheids here are pointed elements, which are very irregular in shape, as though distorted by pressure of the expanded vessel. They are of about the same diameter as the fibres, but differ from them in being shorter, thinner walled and conspicuously covered with pits of the same type as on the vessels.

In some types of wood, e.g. lime (*Tilia vulgaris* Hayne) and horse chestnut (*Æsculus Hippocastanum* L.), a new feature is seen in the vessel elements, where, in addition to the normal pitting of the walls, there is an inner spiral thickening which shows no correspondence with the previous pitting in the secondary wall. In still other types, e.g. birch (*Betula pendula*) the vessel elements differ from those of apple in that the longer and more sloping end wall of the element is developed as a scalariform perforation plate in which numerous narrow, horizontally extended perforations are separated by bars of wall material.

The Annual Increment of Wood.

Now that we have examined the different types of element present in the wood, we can visualise from the transverse

section the kinds of elements which have been cut across. We realise that the vessels lie amongst long, pointed-ended fibres and elongated files of wood parenchyma cells. The files of wood parenchyma cells and the vessel elements are usually of about the same length and, if the comparison is made, will also be found to be about the same length as the long cambium cells from which originally all these wood elements have been formed by tangential division. But the fibres in apple are two or three times this length, and it is difficult to see how these have been derived from the shorter cambium cells. The increased length is usually stated to be due to "sliding growth," by which the young fibre cells are assumed to grow longer and longer whilst their points slip past one another. It seems possible, however, that the young fibres elongated under compression, as at this stage they are undifferentiated cells with plastic walls and liquid contents. Such structures cannot be appreciably reduced in volume, so that, as they develop amidst expanding, growing tissues, the future fibres are compressed and elongated until they attain their final length. Whether, during the process, the point of a fibre slips amongst its neighbours, or whether the whole framework of walls of the future fibres deforms to form a more elongated system with interlocking points, remains to be determined.

If we now take as an example of an older woody stem, a transverse section of a stem of lime, about four or five years old, the most obvious change is the marked increase which has taken place in the ring of wood (Fig. 88). In this wood the annual increments of growth, or annual rings, are clearly visible, though some analysis is necessary of the differences in the wood structure which makes these rings visible. All the wood ring is composed of elements of the same general types, but in the early wood the wood vessels may be larger, whilst in the outer part of the wood of the annual increment the vessels are narrower, and lie amongst elements with smaller cavities and usually with thicker walls. The contrast of the last wood formed one summer with the early wood formed the next spring is sufficiently marked to give the impression of a ring visible to the naked eye. In some woods the most conspicuous feature drawing attention to successive annual rings is the large size of the spring vessels when the wood is described as ring porous (Fig. 87a); in other woods the

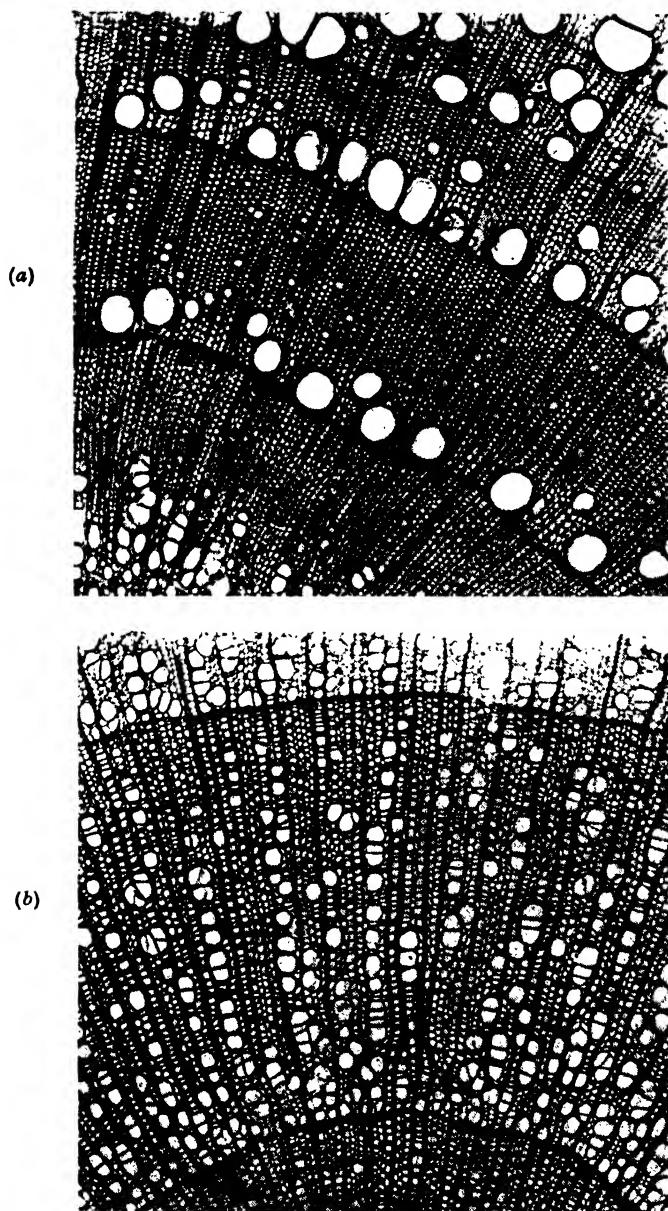


FIG. 87.—(a) Ring porous wood of ash ($\times 30$), (b) diffuse porous wood of alder, in transverse section ($\times 50$).

vessels are distributed more uniformly throughout the ring and show no sharp change in diameter from early to late wood. In such diffuse-porous wood the rings may be sharply indicated by the close texture of the outermost elements of the late wood (Fig. 87*b*). The later formed wood is often termed autumn wood, but wood formation has often ceased for the year in early summer, so that late or summer wood seems a more appropriate term.

Radiating through the wood the rays are now very conspicuous. Some of these can be traced right out from the pith to the periphery of the phloem; these can usually be seen to reach the pith on the flanks of one of the original leaf trace bundles and would thus legitimately be called medullary rays. Many of the rays, however, cannot be traced so far as the pith, and it is clear that the shorter distance a ray can be traced inwards the more recently must it have arisen, and it will be noticed that, whilst some rays extend from pith to cortex, many of the narrower rays which run a shorter distance into the wood also die out before they have run far out into the phloem. If the distribution of the rays is studied, it will be seen that, although the pattern is not very symmetrical, yet two rays never diverge far from one another before a new ray appears between them. Consequently as the periphery of the wood grows larger, rays are as frequent as when the periphery was much smaller (Fig. 90). It is necessary to visualise what the appearance of a new ray means. In a tangential longitudinal section through the wood or cambium, the ray is seen as a short file of parenchyma cells (Fig. 89*a*), with the long wood or cambial elements running down each flank. Evidently then as the sheet of cambium grows in surface, new rays appear in it, at points as far distant as possible from other rays. The new ray originates through the transformation of a long cambium cell into a vertical row of typical vacuolating and dividing cells by a series of transverse divisions. When once formed in the cambial ring, such ray initials never disappear, but continue to grow and divide so that the rays form long spokes of vacuolated tissue which radiate through wood, cambium, and phloem. Though the contents of the long cambium cells are not very dense and appear to have vacuoles, these do not behave like typical vacuoles and the cells remain closely pressed together without intercellular spaces between them as in the case of eu-meristem; nevertheless these

cells need oxygen for their vigorous growth, and it is an important characteristic of the rays that, as they consist of normal vacuolated cells, they are always associated with intercellular space systems (Fig. 89*b*). These systems run out along the rays continuously through wood, cambium, and phloem, and in course of time they come into communication with air spaces in the cortex to the outside of the phloem. How this comes to be so, in the case of the later formed rays, needs some consideration, as when first formed they appear to end in the phloem.

In the transverse section of lime the phloem is very striking in appearance. It lies in wedge-shaped groups (Fig. 88*a*) which taper towards the outside, whilst the rays expand between the phloem wedges. The secondary phloem itself is differentiated into alternating bands of thick-walled, lignified fibres and thin-walled tissue consisting of sieve tubes, with their companion cells, and phloem parenchyma (Fig. 89). The thick-walled fibres are very resistant to pressure and soon lose all power of growth, so that, as the stem periphery

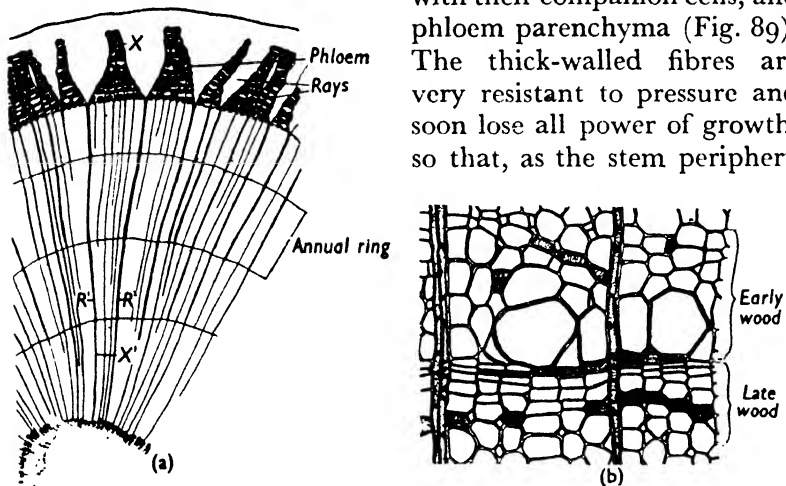


FIG. 88.—Lime. (a) Diagram of part of a transverse section of the stem to show how the annual increment of wood and bast modifies the structure ($\times 12$), (b) the delimitation of the annual ring ($\times 160$).

increases, they retain their original dimensions unaltered. This clearly must mean that the secondary phloem will taper outwards. For example, the small tangential area of phloem at X in Fig. 88*a* must have been cut off from the cambium when the stem was at an early stage of its radial growth. At that time the cambium would be present at some radial depth X' , where the area subtended by the cambium between the two rays R_1 and R_2 was not greater than the arc of phloem

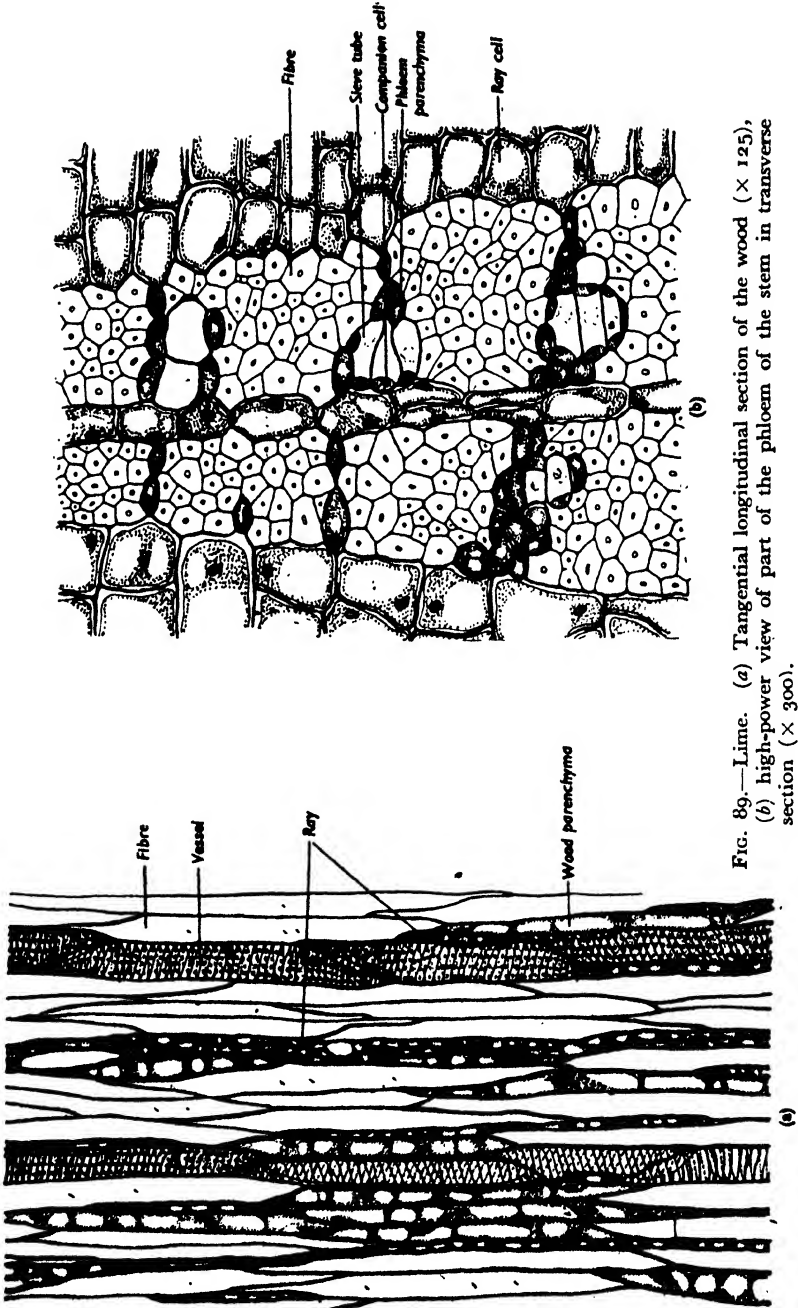


FIG. 89.—Lime. (a) Tangential longitudinal section of the wood ($\times 125$), (b) high-power view of part of the phloem of the stem in transverse section ($\times 300$).

present at X. As the cambium was driven farther outwards by the increasing increment of wood, its periphery gradually increased and the arc of phloem cut off between R_1 and R_2 increased proportionately, and as this widening process continues as the periphery of the cambium increases, tapering wedges of phloem must result. In the cambial ring the ray cells do not divide by radial longitudinal walls and do not widen appreciably in the tangential direction. The cells cut off to the outside by the ray initials immediately come under the stresses which are inevitable in this part of the stem, which must increase in periphery, and in which

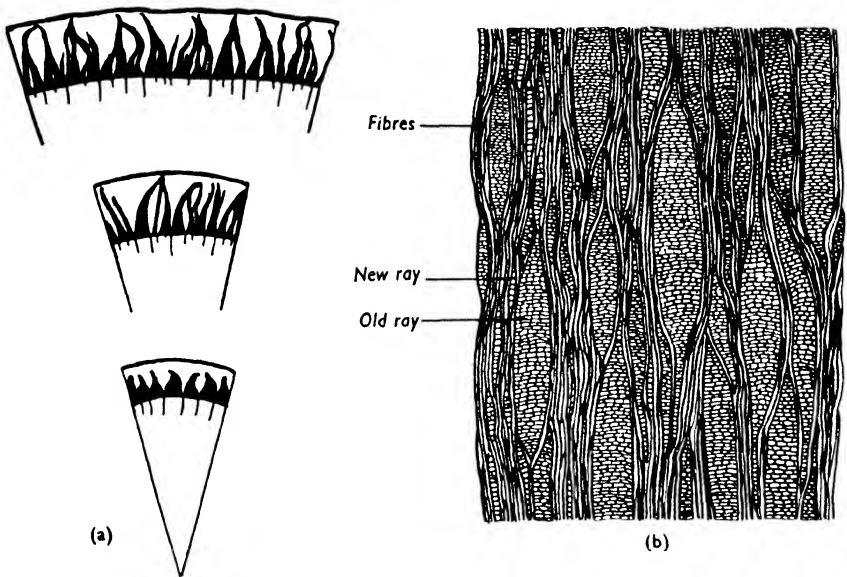


FIG. 90.—Lime. (a) Equivalent portions of transverse sections of 2, 7 and 21-year old stems to show the increase, with age, in the number of tapering tongues of phloem ($\times 4$), (b) tangential longitudinal view of the phloem; the network of phloem fibres continues to be torn apart with increase in girth ($\times 20$).

the phloem wedges are very resistant to expansion owing to the bands of fibres. The cells of the ray in the phloem are living, parenchymatous cells and, as they are driven outwards by the continued growth of the stem, they flatten radially and extend tangentially and also undergo frequent radial divisions, so that the space between the tapering wedges of phloem, which are continually being driven wider apart, is filled by the expansion of the inverse wedges of ray parenchyma.

The interpretation so far is based upon transverse sections, and if further we compare transverse sections of young and old stems (Fig. 90a), we can see in both the same arrangement of widening ray and tapering phloem wedges towards the outside of the stem, but in addition the numbers of such alternating wedges is also increasing. In order to see how this occurs, a tangential section should be cut through the phloem. In this view the expanding rays are seen as broad, biconvex areas, bounded by the sinuous course of the bands of fibres (Fig. 90b). But we also see between the wide rays, narrower patches of parenchyma which appear here and there between the elongated elements of the strands of fibres. These are evidently places where a later formed ray has made its appearance. As the tree increases in age, this earlier formed zone of phloem would be forced farther out, and the network of phloem fibres thus still further strained apart, until eventually the fibres separate and parenchymatous rays develop outwards and become continuous with the loose, tangentially strained tissue to the outside of the stem. In transverse view, a tapering wedge of phloem will thus have been split radially into two, but as each wedge continues to grow at its base, through the activity of the cambium, the appearance of the phloem will not change, and the growth of the parenchyma of the ray will soon fill any transient gap caused by the violent nature of the strains that are thus tearing apart the fibres of the phloem. These features of radial growth are very suggestive in relation to the characteristic oval flakes of bark that are to be seen on the trunks of many kinds of trees.

Softwoods.

We may now turn to compare the wood of the hardwood with that of the softwood. In addition to detailed differences between the secondary xylem and phloem of trees of these two groups, in sections of a softwood numerous resin canals usually ramify throughout the tissues. In this introductory study, however, we must limit ourselves to those points which determine the characteristic differences between the softwood and the hardwood timber, and which serve best to explain the different appearance and behaviour of these woods so familiar to woodworkers.

If we examine a piece of pine stem in transverse section, we see at once that the wood of this softwood has a very different structure from that of the hardwoods. The difference is seen in the great uniformity of the wood, which, apart from the rays, is constructed entirely of one type of element, an observation that is readily confirmed if some of the wood is macerated. The elements are all tracheids, which have conspicuous bordered pits where two tracheids have been in contact by their radial walls, and more crowded pits with narrower borders where they have been next to ray parenchyma. No vessels are present, and that possibly explains why no fibres are present either. The tracheids have a greater radial expansion, wider cavities, and thinner walls in the early wood, and less radial extension, narrower cavities, and thicker walls in the late wood, so that here also annual rings can be recognised.

In the transverse section the rays can also be seen radiating through the wood ; as in the hardwood these are formed by tangential division of short files of vacuolating cells in the cambial ring, whilst the rest of the cambial ring is composed of very long, compressed cambial initials, from which cells are cut off to form the long tracheids. The rays in the softwood are very narrow, in fact seldom more than one cell in width, and they are also comparatively short in the longitudinal direction, so that they are not readily recognised when the wood is examined by the unaided eye.

Wood Blocks. Hardwood and Softwood.

Many of the features of wood may be seen readily by the examination of blocks of wood under the hand lens. For the hardwood a block of oak is very suitable with three polished faces in different directions, transverse, longitudinal radial, and longitudinal tangential. In the transverse view of this ring-porous type, the annual rings are very conspicuous owing to the contrast between the early wood, with the wide cavities of the vessels, and the even grain of the late wood. The broad rays are also conspicuous and are often termed the silver grain, as the light reflected back from the walls of the ray cells, which lie near the surface in this view, gives to the rays a silky, almost silvery appearance. If the rays are followed on

to the other faces, in radial view they have the same silky sheen and can often be followed for some distance before the cut passes into a slightly different plane, but in tangential view, when the rays are cut across the long axes of the cells, the deeper cell cavities do not reflect the light in the same way and the rays appear as long, thin dark streaks. On the radial face the alternating bands of spring vessels and of fibres give a long straight grain to the wood, and the vessels which have been opened in the cutting look like long, narrow channels in which, with the aid of a hand lens, the constrictions due to the perforated cross walls may be seen. If a splinter of oak wood with such opened vessels is examined under the microscope with reflected light, the pitting on the vessel walls shows that these have been surrounded by groups of much-twisted tracheids, which have evidently been distorted by the great expansion of the vessel during development, and also by bands of ray cells elongated at right angles to the direction of the vessel. (In many samples of oak wood the cavities of the vessels become filled by balloon-like growths, which are really proliferations through the pits from the living cells around. When the cavity is filled by such thyloses the sample is obviously not suitable for examination by reflected light.) On the tangential face the grain, due to the arrangement of early and late wood, is much more puzzling at first sight, but the varied appearance is not difficult to understand if the observer remembers that the straight saw cut cannot travel for long down the same ring of wood, but will pass from the early wood of one annual ring into the late wood of the next ring and so on. As a result the zones of early and late wood will form patterns of curving bands in the more central region of the tangential face, and straighter lines on either side as the cut, strictly tangential in one region, becomes more radial on either side.

The softwood block is best prepared by sawing and polishing a transverse, a tangential, and one radial face, the other radial face is split along the natural planes of cleavage of the wood. In transverse view the annual rings show very clearly, due to the fact that the early wood is much lighter in colour than the late, gives more under the saw, and also cracks and contracts more on drying. Closer examination under the lens shows that the early wood in this case is not

characterised by vessels with wide cavities, in fact the pores of the minute tracheids, even in the early wood, are too small to be visible even under the lens. Under the microscope the tracheids are clearly seen in their regular radial rows, but their average diameter is only about 40μ or 0.04 mm., whilst in oak the diameter of a spring vessel may be as much as 0.5 mm. A further difference is that the rays are not visible in transverse view with the naked eye, though with a hand lens it is just possible to recognise them as very delicate lighter lines running through the darker, late wood. The rays are also practically impossible to see on sawn faces in radial or tangential longitudinal view, but on the face which has been split, the cleavage tends to travel along the rays, which can be seen as small, horizontal bands of light-coloured cells. A splinter off the radial surface, examined under the microscope by reflected light, shows the individual tracheids, each with a line of large, bordered pits along its length, and also bands of horizontally running ray cells. The silvery appearance of the tracheids in the dry block is due to the presence of air in them. If a drop of alcohol is placed on the surface this rapidly displaces the air and the tracheids soon become the same colour as the rays. On the various faces the patterns made by the early and late wood are very similar to those in the hardwood. Floor boards are usually made of softwoods and the characteristic patterns of the radial and tangential cuts can be recognised. The thin-walled early wood becomes much more worn than the thick-walled late wood, so that the late wood is left in raised patterns on the planks.

If the block of oak is examined in surface view, the annual rings will be seen to vary in width, and the impression is received that the broader rings owe their greater width to the formation of relatively more wood of the late type. In the pine, on the other hand, the wider rings show the presence of relatively more of the thin-walled, early wood. This contrast suggests that in ring-porous, hardwood trees like oak, stronger timber will be produced by trees grown under conditions favourable to the production of wide rings. In softwoods the converse is true, and the narrower the annual ring the greater will be the proportion of the more durable late wood. Softwood timber sufficiently strong to give satisfactory pit props or good durable planks may thus be obtained if the trees have been

grown in situations where they have made comparatively little growth each year. Soils suitable for the growing of hardwood timber may often be used to better advantage for the growing of crops which mature more rapidly, but poor soils in exposed situations may be used for the growing of softwoods, as such soils would not produce crops of other kinds sufficiently well to repay the cost of labour, etc. Thus the less closely populated lands of the warmer regions of the world supply much hardwood timber, whilst large supplies of softwoods come from the sub-arctic regions.

Another feature is seen in pieces of laburnum wood which have been cut transversely and polished. In relatively small stems the full compass of the annual rings from pith to bark can be included in the section, and it will be noticed that the inner rings are of a very different colour from the outer. In both hardwoods and softwoods this distinction between inner heartwood and outer sapwood is often present, though not necessarily so obvious. The inner rings are of course older, and it seems that, even during life, this region tends to become progressively drier, and to some extent air permanently replaces water in these older tissues. Air in the wood is very important to the lumberman in places where logs have to be floated down a river to the mill, because the air makes the wood more buoyant and, moreover, it is not easily driven out again even when logs are immersed in water, so that logs with heartwood are more likely to reach the mill whereas those without heartwood often fill with water and sink. In laburnum, as in many other kinds of trees, the drying of the older wood is accompanied by changes in the chemical nature of the walls, etc.; these changes probably begin in the living cells, in which starch disappears and resinous and fatty substances are formed instead, which impregnate and discolour the wood.

Applications of Comparative Anatomy.

In the chapters dealing with anatomy it will be found that special plants have been selected to illustrate particular tissues and types of tissue element and it is important to realise that, although we can recognise the equivalent tissues in different

plants, the detailed arrangement of the tissues and certain features of the constituent elements are often characteristic of the plant under examination. Consequently it is usually possible to determine the kind of tree from which a wood sample has been taken by examination of a small fragment of the material and similarly the constituent plants of herbal mixtures, etc., can often be recognised from the special features of small parts of leaves, bark, etc. This branch of Botany has therefore considerable application in relation to police investigations and certain commercial disputes where the nature of a plant product is in question.

It is also becoming more and more evident that anatomical evidence may be of considerable value in determining the relationships of one plant to another where morphological evidence is inconclusive and in identifying plants in tropical jungles where the flowers are inaccessible. As our knowledge of anatomy becomes more complete, its value to the systematic botanist is likely to become more and more important.

CHAPTER XXV.

THE RADIAL GROWTH OF THE TREE.

So far we have merely considered the structure of the woody stem, and any conclusions as to its mode of growth have been based on facts of structure. We must next review briefly the way in which the new increment of tissue is added when the season's growth begins. We are now considering, not the first year shoot, but any older woody stem in which growth in length has ceased and which, in the English climate, will have spent at least one winter in a dormant condition. Next spring the cambium layer will once more resume activity, and will divide vigorously by tangential divisions to form the cells which will differentiate into the wood and phloem of the new season.

The renewal of growth in the cambium can be detected by the fact that the bark slips and separates readily from the old wood. When the bark slips in this way, it has torn away from the surface of the wood at the expense of the cambium, a meristematic layer with liquid protoplasmic contents and thin walls. When this layer recommences growth in the spring, the first new tissues formed from it are cut off to the inside and, after separation of the bark, are left as a soft layer over the surface of the hard, lignified wood of the previous year. They can, therefore, be scraped off in thin strips suitable for microscopic examination, and in which the outlines of rays and long pointed "fusiform" cambial elements will be faithfully reproduced, because these cells are replicas of the cambial cells from which they were cut off by tangential longitudinal divisions (Fig. 91). No simpler or more successful method than this can be adopted to obtain preparations to show the appearance of the cambium as seen in tangential longitudinal view, and in which also early stages in the differentiation of the xylem may be followed. The strips need to

be taken soon after cambial activity has recommenced ; later the depth of tissue thus removed from the old wood is too great to permit of satisfactory study.

In the spring, in practically any tree, as the buds begin to swell, the bark first slips on the twigs just beneath the buds, and strips of tissue can first be obtained by the above method from the surface of the wood in this position. The renewal of cambial activity then spreads from the buds, downwards throughout the whole tree, from twigs to branches to trunk, and even to the proximal regions of the root system. The

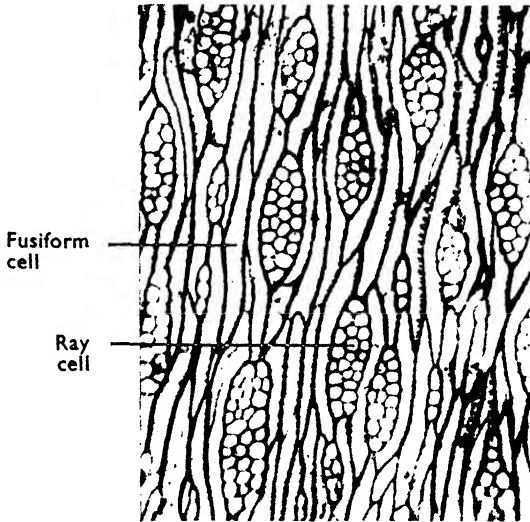


FIG. 91.—Photograph of a strip of differentiating tissue from the surface of the old wood of ash in spring, showing the pattern of fusiform and ray cells ($\times 100$).

significance of the buds in the renewal of radial growth was noted by a German forester, Th. Hartig, in 1862, but the observation was neglected. It has recently been confirmed by many observers, and is probably of the first importance in the understanding of the growth habit of the tree. Thus it has already been pointed out that fresh light is thrown, from this standpoint, upon the normal horticultural procedure of pruning shoots back to just above a bud and, upon the practice in forestry of close planting.

By close planting the forester delays the growth of lateral branches and encourages their natural abscission. In this

way he has developed the forest tree with a crown of branches carried aloft upon a long shaft or bole free from branches. By mutual shading the growth of buds along the length of this straight shaft is prevented, and it follows that the impetus to cambial activity, which is to travel down the trunk, must be started in the first place by the growth of the buds on the twigs, which form the ultimate ramifications of the crown. This very remarkable growth habit is one of the essential characteristics of the tree. The impetus to renewed growth often travels down the main shaft astonishingly fast when it reaches it, but it is not surprising that radial growth is often several weeks later in starting on the trunk, as compared with the first year shoots. Furthermore, each year the food utilised in this growth in thickness of the main bole has to be derived from the supplies made by the activities of the leafy crown and, as the proportion of shaft to crown increases, a time will come when the food supplies thus available do not maintain the same vigour of cambial activity from the base of the crown to the base of the trunk. The result is usually an increment of wood which becomes thinner towards the base, and this leads gradually to the development of a bole which, instead of tapering gradually from the base, has about the same thickness for many feet from the base of the tree. Such a tree is ideal from the point of view of the forester, for out of this symmetrical shaft long planks can be cut with the minimum of waste, but when the trunk attains this form the tree is becoming ripe for felling. We have yet to consider the root system of the plant, but it is obvious that its growth must depend, like that of the trunk, upon the supplies from the leafy crown. When therefore the trunk is making proportionately less growth in the basal regions, the root system is also probably making less growth below ground, and it is this root system which takes up water supplies from the soil and contributes to the vigour of next season's burst of foliage. As the tree reaches the symmetry desired by the forester, therefore, the altering balance of growth seems to mean, in total, less leaf and less root and less timber production per tree. The tree will not be contributing so good an annual increment of wood, so that the time has arrived when it will probably be the economic policy to fell it.

The Functional Significance of Radial Growth.

The seasonal renewal of radial growth in the tree must have a very important bearing upon the functional activity of the tree. During the winter very little growth activity has been in progress, though below ground the roots may have continued growth for some time, and certainly they have continued to absorb water, as the water content of the wood rises in winter.

The increased water content of the tree, associated with the rise of temperature in the spring, probably influences the recommencement of growth from the buds. An experiment we owe to Sachs is very suggestive in this connection; pieces of sycamore stem, about a foot long, are cut from the tree in March and one end trimmed smooth with a razor, the pieces are then plunged into hot water, with the trimmed end protruding, when sap will well out of the wood, coming usually from the outer rings first. If the stem is transferred to cold water, the sap sinks back into the wood surface again, and the whole observation may be repeated many times. The same effect can be produced if the stem is simply warmed with hot air and is not connected with the entry of the hot water into the stem; it is simply due to the expansion of the water already present in the wood which, as movement takes place most readily in a longitudinal direction, soon appears at the cut ends. The same expansion of liquid will take place in the intact tree when the temperature rises in spring, and the liquid will move up the longitudinal channels in the wood. In all the old rings of wood these channels, from their method of production, must now be blocked where they terminate at old leaf or scale scars, but in the last year's wood, a number of the channels will run into the buds on the one-year twigs. Thus when the sap in the tree expands, it will be driven into the xylem vessels running into the buds and this may well be responsible for their renewed growth.

Each bud as it recommences growth is soon subtended by a wedge of newly-developing wood upon the surface of the old wood (Fig. 93). The new wood may be removed and examined as strips by the method previously described (Fig. 92). By this method all the new tissues are removed from the old wood surface when they are still only a few cells deep, and in such a layer it is possible to follow the same vessel in

the preparation for a considerable distance, even those in very early stages of differentiation. One remarkable fact then impresses the observer, however far the same vessel is followed in this way it is impossible to see any difference in

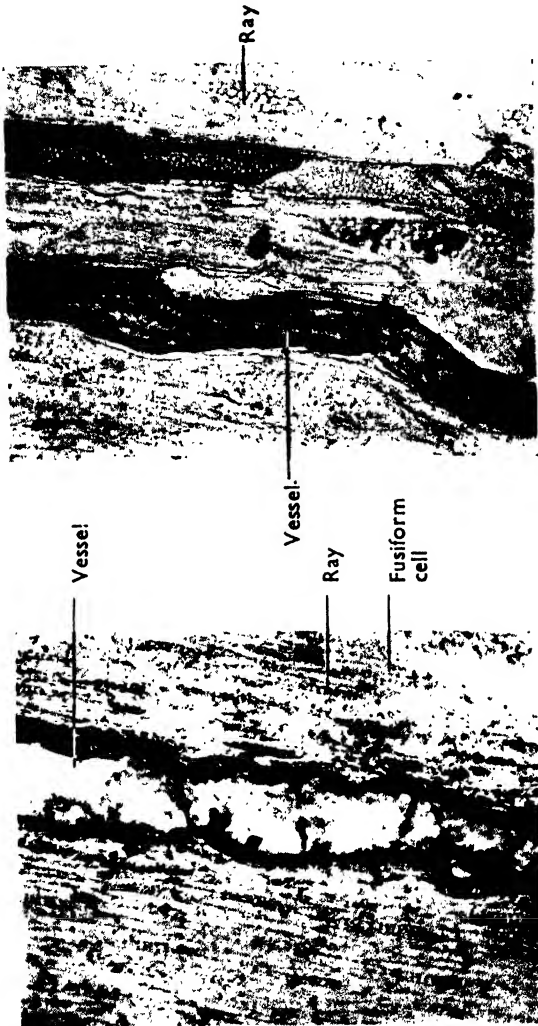


FIG. 92.—Photographs of stained strips of differentiating tissue removed from the surface of the old wood in spring ($\times 230$). (a) *Castanea*, the young vessel still has contents, the cross walls are perforated, but the walls are not thickened, (b) *Sycamore*, the vessel walls are thickened and lignified.

its stage of development throughout that length. It thus appears that the vertical differentiation of the same vessel must proceed with great rapidity, a fact that becomes the more remarkable when it is added that in the ring porous trees these vessels are of extraordinary length; it seems

possible indeed that the same vessel may be continuous throughout the length of the main trunk. As these vessels are also of comparatively large diameter, they may be seen very clearly after the bark has been peeled off, if the surface of the exposed new wood is allowed to dry, when the vessels stand out prominently on the surface. The same method has been used, in Fig. 93, to show how the new and smaller vessels spread fanwise over the old wood from the base of a bud upon a willow cutting as it recommences growth.



FIG. 93.—Willow, vessels differentiating from the base of a bud, rendered prominent by allowing the tissues to dry ($\times 3$).

The newly differentiating vessels must expand with great rapidity throughout their whole vertical course. They lie upon the surface of the old wood, and there can be little doubt but that the water for their expansion is withdrawn from the old wood, in which it has been accumulating all the winter. As the vessel expands, the transverse walls of the constituent cells are unable to stand the strain; they tear and retract immediately to a narrow rim, which in the strip preparations is seen as the only thick portion of the wall of the vessel at this very early stage of its differentiation. Although the vessel is already, at this stage, a cavity with one common cellulose boundary, its liquid contents are still contained in the long file of separate protoplasts of the original cells. These can be demonstrated by plasmolysing the young wood, taking pre-

caution not to damage the vessels before doing so, by immersing its surface in very concentrated salt or sugar solution, and subsequently examining the vessels in tangential slices mounted in the same solution (Fig. 94).

The protoplasts in the vessel elements now commence to deposit the layers of cellulose of the secondary wall upon the thin primary wall of the vessel, and this file of protoplasts persists until the wall of the vessel is finally thickened and lignified. Some time after the vessel wall has thickened and lignified, the protoplasmic contents will be found to have dis-

appeared, presumably they have been in part exhausted in the process of wall deposition and lignification. Their disappearance means that the liquid contents of the vessel are no longer retained within the file of semipermeable protoplasts, and the question arises as to what will happen to these contents. The answer seems clear, for this vessel is not only differentiating in a



FIG. 94.—Photograph of vessel elements of ash, with plasmolysed protoplasts within ($\times 130$).

continuous path over the old wood from beneath a growing bud, but it is also continuous upward into the axis of this bud, and is at this end in close contact with other vessels which run right into the leaves expanding from this bud. It is clear indeed that this astonishing vessel development in the tree is only a further continuation of the process of differentiation of primary xylem from young leaf and extending internode into adult internodes below as secondary xylem, which was

followed in *Helianthus* in Chapter XXII. The vessels in the leaves of the bud are supplying water and solutes to actively growing and transpiring leaves; evidently then the supplies, now released by the disappearance of the protoplasts in the differentiating vessels over the surface of the old wood, will find their way up to the leaves in the expanding buds.

The vessels differentiating in the wedge of new tissue beneath the buds are thus seen to function as a system, which first absorbs water from the old wood beneath the cambium and then releases it into the leafy shoots which grow out from the buds, and the early spring vessels are probably very important in this connection. During summer, when the foliage is fully expanded, much water is lost from the tree by transpiration and at the same time the water table in the tree is falling. Obviously under these conditions the water supply to the foliage is likely to involve other physiological processes than those just discussed, but these are best deferred until the absorption of water by the root has been studied and the question of water balance can be discussed as a whole.

It is clear that the altering conditions of supply, of water and food, should affect the type of elements differentiated during radial growth. Later in the season when the foliage is fully expanded, a condition reached in most trees within the first month or two from the opening of the buds, fewer cells are cut off from the cambium to the inside and these differentiate into the late type of wood; to the outside cells are being cut off fairly rapidly and these, during the summer and even into the autumn, differentiate into phloem, which commonly consists of groups of sieve tubes with companion cells and parenchyma, alternating with groups of thick-walled bast fibres. We have very little information as to the longitudinal direction in which the phloem tissue differentiates, but there can be little doubt that its differentiation is associated with the movement of food substances out of the adult leaves, and there is every probability that it differentiates downwards. Evidently every new element that is formed in such a chain as it grows, receives supplies from a leaf above, and represents, in its own formation, a movement of those food supplies downwards. During the summer the growth of the phloem is very vigorous, and the amount of downward movement of food represented by its

growth alone must be very considerable. In addition to this, food must be lost from the differentiating sieve tubes in considerable quantities for, as each protoplast of a sieve tube element disorganises and loses its semipermeability, the solutes will be released to the surrounding tissues. Perhaps it is by this channel that the carbohydrates reach the developing xylem elements on the inner side of the cambium, where they are converted into cellulose and deposited on the walls of the late wood. In this form, and as the starch that now fills the parenchyma cells of wood and phloem, most of the excess food supplies from the leaves are stored during the summer.

In the trunk of the tree the chains of elements that constitute the sieve tube must reach very considerable length, and in this region also the individual sieve tubes attain remarkable dimensions. The phloem elements are best seen if longitudinal sections from the trunk are macerated in chromic acid and stained in hæmatoxylin. Material of ash or elm may serve as an example of a type with the simpler form of sieve tube. The sieve tube elements are recognised by the fact that they are wider than the other kinds of element present and appear as short, wide cylinders with the ends transverse or only slightly oblique. When the end wall is slightly tilted, it is seen that all that remains of the cross wall is a network of strands of thickened cell wall, the perforations in which are very large in comparison with those in the majority of herbaceous plants (Fig. 95*a*). A transverse sieve plate of this kind is described as a simple plate. On the longitudinal walls of the same elements various patterns of pitting are seen, either as rounded pits or as a series of transversely extended pits with the thickenings between them forming a network; such pits correspond between two sieve tubes or between the sieve tube and the phloem parenchyma or companion cells, but the thin areas in these cases are not broken down into actual perforations. In addition to the sieve tube elements, the macerated material shows companion cells (which are frequently subdivided into a chain of cells), phloem parenchyma cells, and phloem fibres.

If the phloem of oak or lime is examined in a similar manner, the sieve tube elements are seen to be longer and relatively narrower than those of ash and to have sieve plates of the kind described as compound. The end wall forming the plate

is long and sloping, and the perforated region is divided up into a number of areas by transverse bars of thickened wall. There may be seven or more of these areas on which the network is clear, but of a finer mesh than in ash

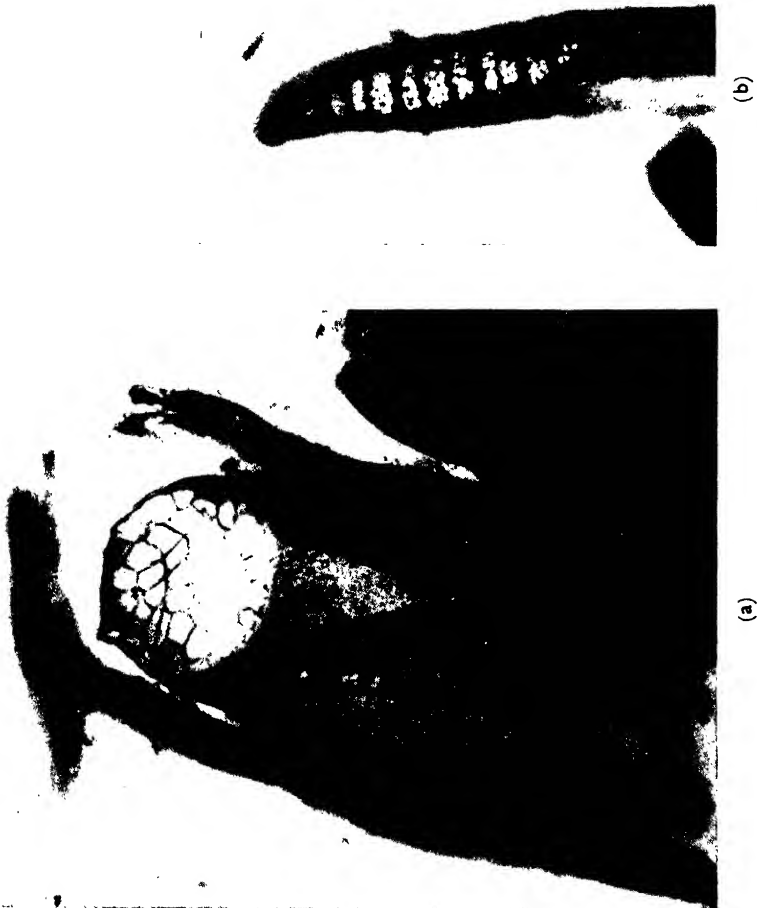


Fig. 95.—Photographs of sieve tube elements from macerated phloem of the trunk of, (a) common elm, (b) lime ($\times 400$).

(Fig. 95*b*). Some of the compound plates may lie in such a way that they can be seen in optical section, when the bars of thickening appear as beads, with the thin perforated regions between them. The pitted regions on the longitudinal walls are also very distinct.

Ringing and Girdling.

In the brief statement of the manner in which xylem and phloem differentiate in tissues derived from the cambium, it has been pointed out that the mode of development of these tissues is in accord with a movement of water upwards through the xylem into the expanding shoot, and a subsequent movement of manufactured food substances downwards from the fully expanded leaves. Though the general question of water movement is deferred until a later stage, a little further light is thrown upon food movement by the results of the processes known respectively as ringing and girdling.

By ringing is meant the removal of all the tissues down to the cambium over a complete but narrow area of the circumference of the stem. The result of the process varies with the kind of tree, and the extent to which precautions are taken to prevent the drying of the wood thus exposed. When a ring is made in spring, there occurs above the ring a vigorous swelling which is mainly due to the formation of additional tissues, which include swollen, proliferated cells projecting from beneath the bark and which are spoken of as callus. The cambial activity which receives its impetus from the activity of the leafy shoots above, which also supply the food necessary for continued growth of the cambium, is transmitted down as far as the ring, where the cambium is interrupted. Here new cell formation cannot continue downwards immediately, and seems therefore to continue as an increase in the vigour of radial growth ; if the surface of the exposed wood is not too dry, the cambium may succeed in regenerating across the ring when once more cambial activity continues downwards over this region. Until this regeneration takes place, however, there is considerable evidence (in starch distribution, accumulation of carbohydrates in fruits, etc.) for accumulation of food supplies above the ring and a comparative shortage below. Such ringing experiments have, therefore, been regarded as evidence of the fact that interruption of the phloem stops the movement of food, and therefore as support for the view that the phloem acts as a channel for food distribution. This argument has been further strengthened by ringing experiments on plants which have strongly developed internal phloem between xylem and pith, in addition to the

normal phloem, for in these cases the downward movement of food seems to be less seriously affected by ringing.

The leaves above the ring usually remain turgid, so that such experiments have also been used as arguments in favour of water movement in the wood, which has not been interrupted by the ring. We should notice, however, that a usual result of ringing is to force into vigorous growth the buds below the ring, even those which would normally remain dormant. These grow very vigorously like the "water" shoots that often spring from near the base of many trees, and it would appear that these have easier access to the water supply than the less vigorous shoots which develop from buds above the ring. It is clear, however, that so long as water is present in the old wood above the ring, this will be withdrawn by the xylem systems differentiating downwards from the expanding buds.

A very suggestive and simple experiment upon the effect of ringing can be carried out in the laboratory in the winter with twigs of willow (*Salix* spp.). These root readily when removed from the tree and so can be kept growing in water. In such twigs it is possible to show that the renewal of activity in the cambium always travels from the buds down the main stem. Thus even when the twigs are inverted after ringing, the main swelling, due to the check on the spread of cambial activity from the buds, still occurs on the side of the ring nearer to the distal buds (Fig. 96).

If the twigs are left standing with the ring under water, the ringed twigs will root much more freely above the ring; in unringed twigs the roots will be found mainly close to the base of the twig. When the rings are under water most of the additional growth activity above the ring seems to be expended in root production, the twig is not swollen above the ring nor is there much indication of callus production.

The presence of a ring through the phloem of a growing tree, especially if it is made at the base of the trunk, will probably be fatal to the shoot system above it in the course of time, especially in hardwood trees, if the cambium fails to regenerate across the ring. In fact the method of girdling trees by removing a comparatively wide ring at the base of the tree through the bast, and usually through some of the sapwood too, is frequently used in order to kill trees of natural

woodland, so that the land can subsequently be cleared of this forest or scrub by burning or felling the dry dead trees. These facts suggest that, whilst the fully differentiated wood may form a channel along which water movement may take place, yet the proper functioning of the whole water-conducting

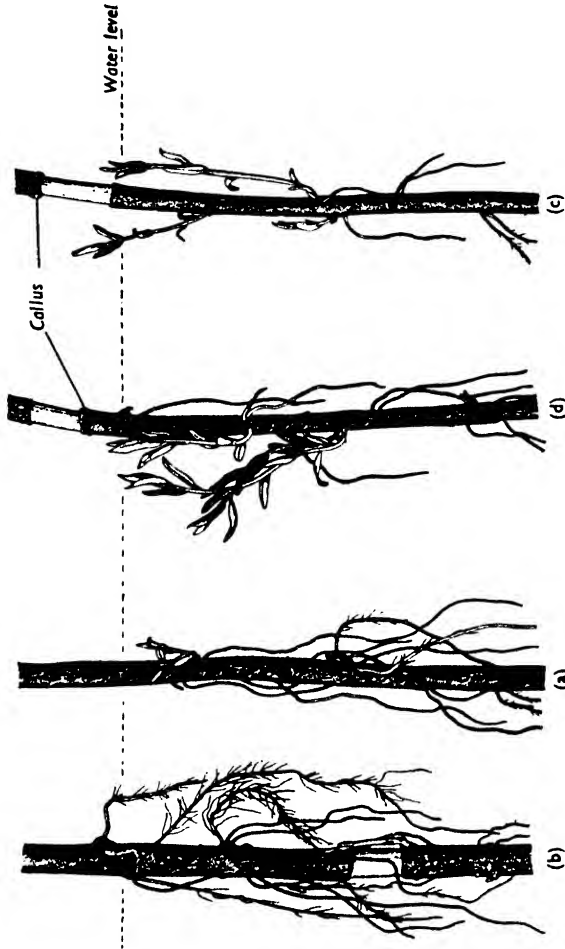


FIG. 96.—Bases of willow cuttings, standing in water, (a) normal, (b) ringed with ring in water, (c) and (d) ringed with ring out of water, and (d) inverted ($\times \frac{2}{3}$).

system and of the food-conducting system of the tree depends upon the continued growth of the cambium throughout the tree, and upon the continued differentiation of new tissue systems from the products of its growth activity. Such a conclusion would be in harmony with the interpretation of the physiological significance of the mode of growth and

differentiation of the tissues which has been given in these pages. It should be emphasised, however, that although only a very inadequate sketch of these processes has been given, yet in the attempt to give a connected account of the growth processes of the tree and of their significance, we have already had to step beyond the statement of ascertained fact to conceptions that are still speculative and as to which there is at present no general agreement amongst botanists.

Cork, Lenticels, and Bark.

In our consideration of the tree we have treated it so far as if it consisted simply of a shaft of wood, surrounded by the layer of living cambium which added wood to the inside and bast or phloem to the outside. But the reader will recollect that this cambial activity in the vascular ring originated in a stem which, like the normal herbaceous stem, was clothed by cortex and epidermis. In the first year of growth a woody twig may have the thickness of a pencil, but as growth continues year after year this may be enlarged to a structure with a diameter of many feet. It is clear that cortex and epidermis, tissues which have normally finished growth relatively soon, must have been modified very considerably during these processes. We have already seen, in a twig of apple, that before the cuticle is cracked by the expansion of the twig, the epidermis is already replaced by the formation of a layer of cork or periderm. In apple this new tissue was formed by tangential divisions of a cork cambium which originated in the epidermal cells themselves. The elder (*Sambucus nigra* L.) provides a clear example of a cork cambium or phellogen which arises in the subepidermal layer (Fig. 97a). As the cork begins to form, the epidermal layer often separates from the cells beneath, and the reflection of light from the air in this space has much to do with the grey sheen that rapidly spreads up the green one-year twigs of elders during autumn.

The differentiation into cork of the cells cut off to the outside of the phellogen takes the form of the development of a lamella of fat impregnated cellulose within the original primary wall of each cell. This is described as a suberin lamella and stains with Sudan III like the cuticle with which it has many properties in common. In particular the suberin

lamellæ are relatively impermeable to water, and thus reduce loss of water not only from the protoplasts within, but also, since the cork cells are closely set together, from the tissues within the sheet of cork. The protoplasts of the suberised cells gradually die and disintegrate after the deposition of the suberin lamella, and the outer cork cells, as the stem continues to increase in diameter, will be gradually stretched tangentially, with a simultaneous reduction of their radial dimensions, until they also, like the cuticle, will be forced to crack and tear apart in places. In some

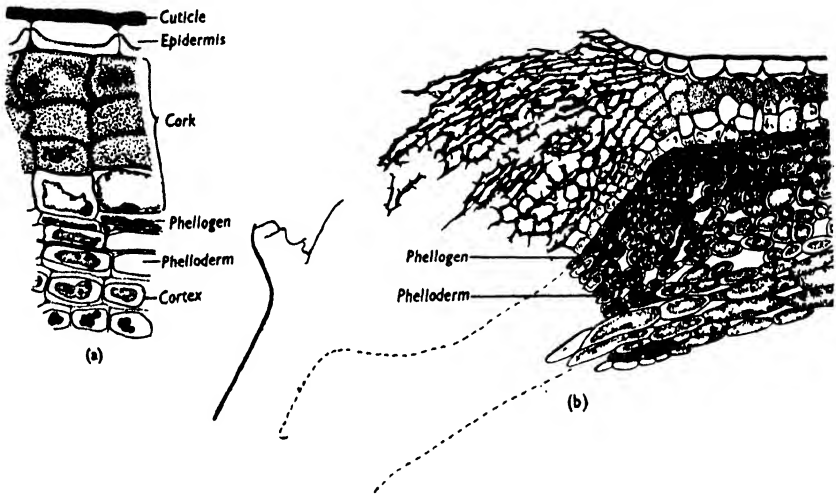


FIG. 97.—*Sambucus nigra*. (a) Sub-epidermal cork as seen in transverse section of the stem ($\times 200$), (b) part of a lenticel in transverse section of the stem ($\times 65$).

plants the living cells of the cork cambium may be able to grow and increase in number so that on the inner side, nearest to the phellogen, the layer of cork cells may still be unbroken. In other cases the stretching and tearing of the first formed cork layer is met by the development of fresh cork cambia, which appear in succession in still deeper lying tissues.

On the surface of the stem of the elder, before cork formation has begun generally, little flecks are visible which pass through their colour changes to grey and brown earlier than the rest of the surface. If these spots or lenticels are examined with the aid of the microscope, sections will show that the lenticel originates below a stoma. In this position

the intercellular spaces are particularly large, and it is in the cells of this loose tissue that the first divisions of the phellogen occur, forming a mass of loosely arranged, powdery cells which push up the epidermis and rupture it, thus giving rise to the cracks in the skin which are seen on the young twig. The divisions then become more regular and a definite phellogen is initiated which spreads out in the sub-epidermal collenchyma until the patches from the various centres join up into a continuous sheet beneath the epidermis. Phellogen activity beneath the stomata begins whilst expansion of the twig is still proceeding, and the new cells cut off to the outside separate from one another and form the powdery mass of cells that can be seen through the cracks in the skin. At the end of the season, when expansion of the vascular ring has ceased, a layer of closely fitting cork cells is formed beneath the lenticel as well as elsewhere beneath the epidermis, but beneath the lenticel the cork is always thinner and thus represents a place of weakness in the continuous layer of winter cork. When growth of the vascular cambium becomes active the following season, with consequent expansion of the twig, the cork sheet is broken through again at these same points, so that the lenticel is filled with alternating zones of powdery masses and ruptured layers of thin cork. In addition to the cork the phellogen may also cut off cells to the inside, which are known as phelloderm. These cells differentiate into normal parenchyma, but may be distinguished from the original cells of the cortex by the fact that the cells have the same radial alignment as the cells of the phellogen and cork. In elder the phelloderm is about five cells deep beneath the lenticels, but usually no more than one phelloderm cell is present elsewhere, and this is really part of the original cell in which the phellogen arose (Fig. 97*b*).

When the same phellogen remains active for long periods of years so that a thick layer of cork is formed, the lenticels are seen as shafts of loose tissue which run through the whole thickness of the cork sheet. Darker rings are also visible running at right angles to the lenticels (Fig. 98); they are comparable with the annual rings in the vascular tissues and represent places where thicker walled and less expanded cork cells were formed prior to the cessation of phellogen activity. The phellogen keeps pace with the expanding periphery, to

some extent by radial divisions, but the dead cork cells to the outside are not able to accommodate themselves in this way and consequently the cork frequently cracks. Lenticels always represent positions of weakness in the cork, and are either developed into deep cracks or may be much pulled out horizontally as in birch (*Betula* spp.).

The external form of the bark is always characteristic of the particular kind of tree and is closely connected with the mode of growth of the cork. New wide sheets of cork may form in continuous layers beneath thin sheets of older bark, which may flake off in wide papery sheets as in birch,

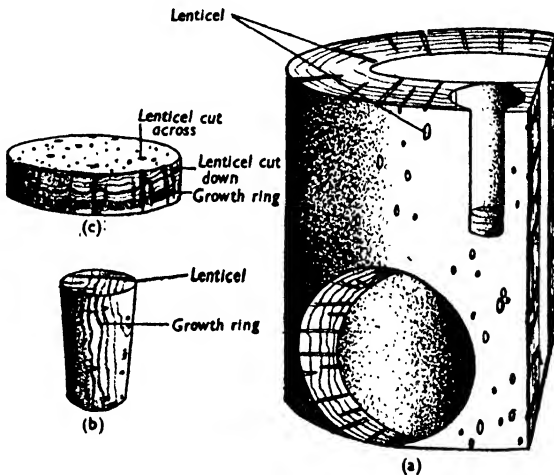


FIG. 98.—(a) Diagram showing the cavities in a cork sheet from which have been cut, (b) cork and (c) shive.

but in many kinds of trees deep cracks penetrate the bark, and the new and successively deeper lying areas of phellogen are also broken in their turn by these furrows. These deep clefts gradually form patterns which are characteristic of the species. The narrower flakes to the outside are composed of the oldest layers of cork, now widely separated from other patches of the same age, and from these points the bark patches or ridges broaden out to their bases, built of younger cork layers still forming from new areas of phellogen, which are continually arising afresh from the living tissues beneath the earlier and fissured layers of cork. The oldest parts are continually being shed as flakes of varying dimensions.

The cork of commerce is obtained from the cork oak (*Quercus suber* L.), an evergreen species of Western Europe and North Africa. Under natural conditions the same phellogen remains active indefinitely and the outer part develops into a rough and deeply fissured bark. This outer region, known as the virgin cork, is of little use for bottle corks and is stripped off the trees when it has grown for some thirty years ; whilst the phellogen is active the bark can be separated easily along this layer. After stripping a new phellogen generates in the cortex, or subsequently in the outer phloem, and the cork is more rapidly formed from these deeper phellogens and is more uniform than the virgin cork, so that after nine years' growth it is usually sufficiently deep to strip again. The process of stripping is usually repeated at nine-year intervals.

As the lenticels run through the thickness of the cork sheet, bottle corks are cut in such a way that the lenticels run transversely across them. The cork sheet is usually not thick enough for very wide corks to be cut out in this direction, but wider, though less water-tight, shives may be cut with the lenticels running vertically through them (Fig. 98). The cork which is not fit for cutting into corks or shives is ground up and used for linoleum, bath mats, and other purposes.

Cork formation also plays an important part in wound healing. In woody plants the scar left by leaf abscission is a corky surface and the artificial wounds made by pruning cuts are healed in the same way. In many herbaceous plants also cork may be formed ; for example as a potato tuber grows the epidermis is replaced by cork and one of the differences between " new " and " old " potatoes is related to the condition of the phellogen. Whilst the phellogen is active the corky skin is easily scraped off, but when the phellogen has ceased activity in the mature tuber this ready separation no longer occurs and the potato has to be peeled. In the propagation of potatoes tubers are sometimes cut into pieces and if these are immediately planted the injured and exposed parenchymatous tissues would allow ready entry of disease organisms from the soil, but if the cut surfaces are exposed to air in a relatively moist atmosphere the injured surface is healed over in two or three days. Fatty substances from the neighbourhood of the wound move outwards and dry at the surface, beneath this blocked surface sap

collects in the intercellular spaces and soon a phellogen arises and produces a sheet of wound cork which provides an effective barrier against the entry of disease organisms. These facts give some guidance to gardeners as it is seen that cut tubers, or cuttings of other plants taken for vegetative propagation, should be placed under conditions favourable to phellogen formation and not under dry conditions when the surface would dry too quickly and crack.

CHAPTER XXVI.

THE STRUCTURE OF THE MONOCOTYLEDON SHOOT.

IN this introductory study it will only be possible to review certain of the most salient differences in the organisation of the shoot of monocotyledons as contrasted with that of dicotyledons. The earlier studies of monocotyledon morphology in Chapter VIII, and of leaf development in Chapter XII, will have made it clear that the differences are very fundamental and initiated at the growing apex, where each new leaf primordium, though arising at a point on the flank of the apex, rapidly spreads tangentially until it rises as a ring-like upgrowth which usually completely surrounds the apex. This deduction from the appearances seen with the lens is immediately confirmed by the use of the microscope, as is illustrated by the two figures which represent the appearance of two typical monocotyledon shoot apices. That of maize is shown in transverse section in Fig. 99a and that of *Tradescantia fluminensis* Vell. in median longitudinal section in Fig. 99b.

In these two plants each successive leaf primordium, whilst still meristematic, surrounds the apex so completely that in *Tradescantia* each appears on both flanks of the apex in the median section, whilst in maize the two margins are already overlapping in the third primordium from the apex. The result is that the differentiation of the leaf trace vascular system, in leaf and axis, commences whilst the leaf primordium is an annular structure around the small central shoot apex, and continues, both upwards into the annular, foliar limb and downwards into the axis beneath, at a time when this whole system is being forced outwards to a more marginal position by the appearance of new primordia on the apex enclosed

by it. In the monocotyledon, even more than in the dicotyledon where subsequent cambial activity may transform the

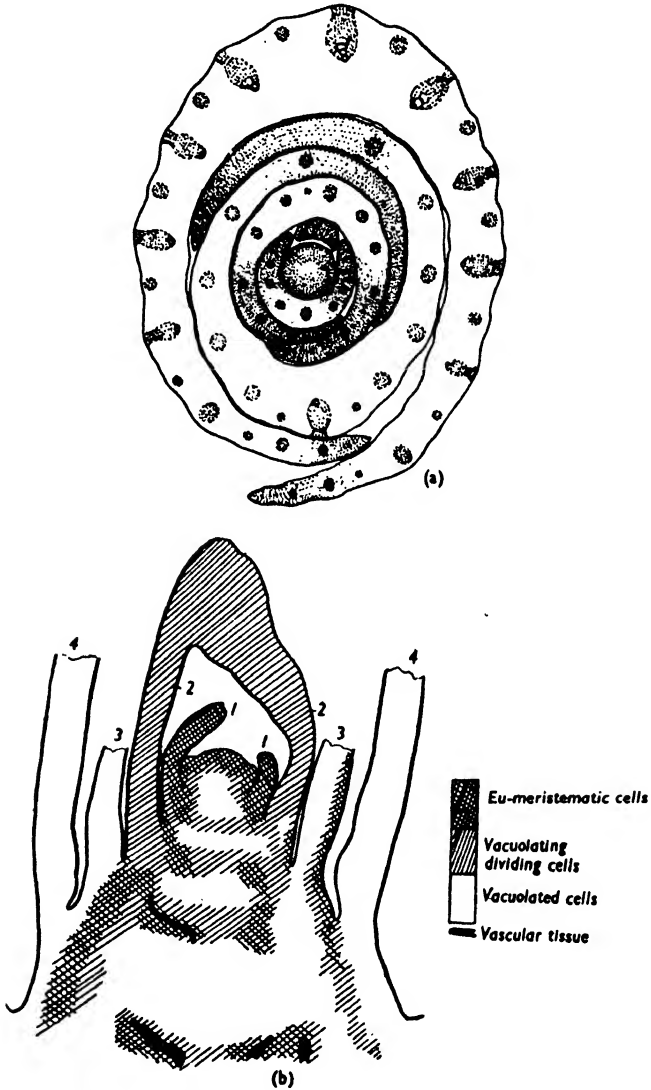


FIG. 99.—(a) Transverse section of shoot apex of maize ($\times 65$), (b) diagrammatic view of longitudinal section of the shoot apex of *Tradescantia*, showing the distribution of the meristem; successive leaf primordia are numbered ($\times 55$).

original structural appearance, the leaf trace system determines the main features of the axial anatomy, and this feature

of development, in which the region of the axis, immediately below and associated with the encircling leaf primordium, must expand and move outwards like a ripple on a pond, needs to be kept in mind when the anatomy of the stem is under consideration. The vascular anatomy of many monocotyledon shoots may become exceedingly complicated, owing in part to the large number of veins which are frequently present in a single leaf trace. In the present study we shall confine our attention to the general characters of the anatomy of the maize leaf, and then consider how the characters of the leaf trace system elucidate certain features of internodal anatomy in maize and *Alstrømeria*.

The Leaf of Maize.

Useful preparations for the microscopic examination of this leaf type are obtained if pieces of maize leaf are first decolorised in alcohol, and then rendered transparent by standing for some days in a saturated solution of chloral hydrate; they are subsequently mounted in pure glycerine. In addition to adult parts of the leaf, the actively growing basal regions may be dissected from seedling plants and treated in the same way.

The most striking features in these leaves are the longitudinally running veins, which form part of the convergent vein system (Chapter XII), but the small section of their course visible in the field of the microscope makes it clear why the term parallel has been used of the venation system. These veins are of different sizes, larger veins with more and larger vascular elements being interspersed between finer veins, in which a few small xylem elements are present. All the veins are linked into a common system by the transverse veins, which join with the larger veins on either side and never end blindly in the mesophyll. In the young basal region it may be possible to see that some of the smaller longitudinal veins and transverse veins are not yet differentiated, whilst the protoxylem of the main longitudinal veins is differentiated right through the growing region. As the most pulled out, annular protoxylem elements are also to be seen above the large scalariform or pitted vessels in the larger veins, it is evident that these large veins are the earliest to differentiate

in the leaf. This evidence as to the course of vascular differentiation in the leaf is supported by a study of vascular differentiation during leaf development.

The main veins, the central one first and then others on either flank, differentiate through the whole length of the leaf and in the axis at an early stage of development, forming the convergent system characteristic of the leaf. At this time the tissue between the veins, and especially at the margin of the leaf, is still meristematic (Fig. 99a), and from this tissue new longitudinal veins are continually differentiated as the lamina becomes broader; the tissue between these veins develops into panels of parenchyma, which are traversed at intervals by the linking transverse veins. All this later vein system develops in the lamina as it matures, and consequently the xylem of the smallest veins is either of a close spiral or reticulate type. The system is completed first at the leaf apex and then develops towards the base of the leaf lamina. Thus it is only when the leaf lamina ceases growth that the latest longitudinal veins to develop are finally differentiated right to the base of the leaf and into the axis.

Although the final development of the panels of parenchyma between the veins thus waits upon the final expansion of the leaf, the cells which then expand and differentiate have originated from the activity of the basal growing region, in which new cells are cut off by repeated transverse divisions. The leaf is also increasing in breadth by the occurrence of longitudinal divisions in this tissue, thus adding to the number of files of dividing cells. The most superficial examination of the leaf tissues shows the remarkable regularity of these longitudinal files.

When the upper epidermis of the adult leaf is in focus under the high power, in surface view it is seen to consist of longitudinal series of rectangular cells with wavy longitudinal walls and, in suitable preparations of growing leaves, these series can be traced into the growing region, where they consist of shorter, straight-walled cells, which are still undergoing transverse divisions. In some of these rows stomata appear at regular intervals; in surface view the guard cells are seen as long narrow cells, elongated parallel to the pore and held between two accessory cells. The regular files of epidermal cells make it possible to trace all the stages in the

process of cell division by which these characteristic stomata are formed, from the stage when the mother cells of the guard cells appeared as alternate, shorter cells in the file, up to the stage when they are compressed between two accessory cells, cut out from the large cells of the adjacent rows

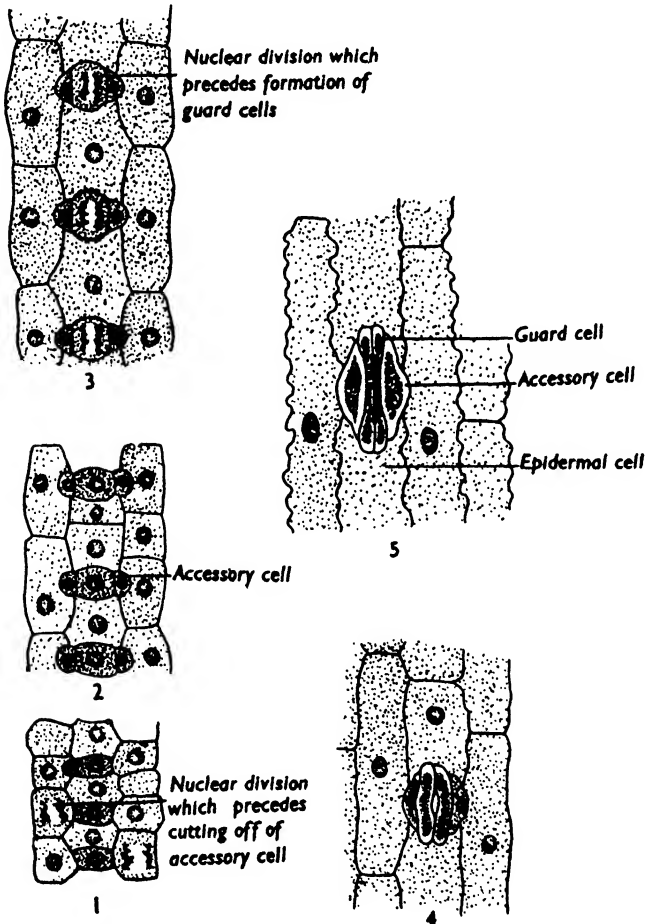


FIG. 100.—Maize, stages in the development of the stomata, as seen in surface view, in cleared preparations ($\times 465$).

of epidermal cells. The mother cell of the two guard cells subsequently divides in the longitudinal plane to give rise to the two elongated guard cells (Fig. 100).

Careful focusing beneath the epidermis will show that the rounded cells of the mesophyll also lie in relatively regular

longitudinal rows, in which, however, the air spaces become very marked in the strips lying beneath the rows of stomata. Over the larger vascular bundles run strands of long cells with thickened walls (only present in fully adult leaves) and preparations with the lower surface of the leaf mounted uppermost show that these are still more marked on this side of the leaf. On the upper surface in well-developed leaves, bands of shorter, broader epidermal cells may be recognised running down midway between the stronger longitudinal veins. Both these latter points can be better elucidated in transverse sections of the leaf (Fig. 101). The main veins are then seen to form solid struts of tissue across the lamina, supported on either side, and particularly on the lower, by caps of thick-walled, lignified sclerenchyma; the smaller intervening bundles lie in the mesophyll, but every bundle is enclosed in a well-marked bundle sheath, which completely delimits the tissue of the bundle from the intercellular spaces around.

The structure of the bundles will be examined in more detail in the stem, but the leaf section shows the position of the protoxylem in the larger bundles as a cavity towards the upper side of the leaf. Below this lie a few lignified elements, but especially prominent on either side is a big vessel, which, when the leaf was in surface view, could be seen to have a close spiral, reticulate or scalariform wall pattern, from which it is evident that these large xylem elements completed their differentiation relatively late in the growth of the leaf. The phloem of the bundle lies below the xylem, between it and the bundle sheath.

The wide epidermal cells, seen as bands on the upper surface between the stronger longitudinal veins, are now seen in the leaf section as groups of cells developed considerably in depth at right angles to the surface. These large cells readily lose or gain water, with consequent changes in form which have the effect that the leaf lamina tends to unroll when the cells are turgid, and to roll inwards when the leaves are losing water; hinge or motor cells of this kind are much more developed in many other grass types, and as they are often associated with the restriction of stomata to the upper side of the leaf, this is a xeromorphic feature which may be of significance in reducing water loss under dry conditions (Chapter XXI).

Another feature that may be noticed about this leaf section,

in contrast with the typical dicotyledon lamina, is that not even the main veins project as prominent ridges on the lower surface of the leaf. In the larger leaves the middle region of the leaf appears as a thick midrib region, but in section it is seen to contain a large number of veins, which lie towards

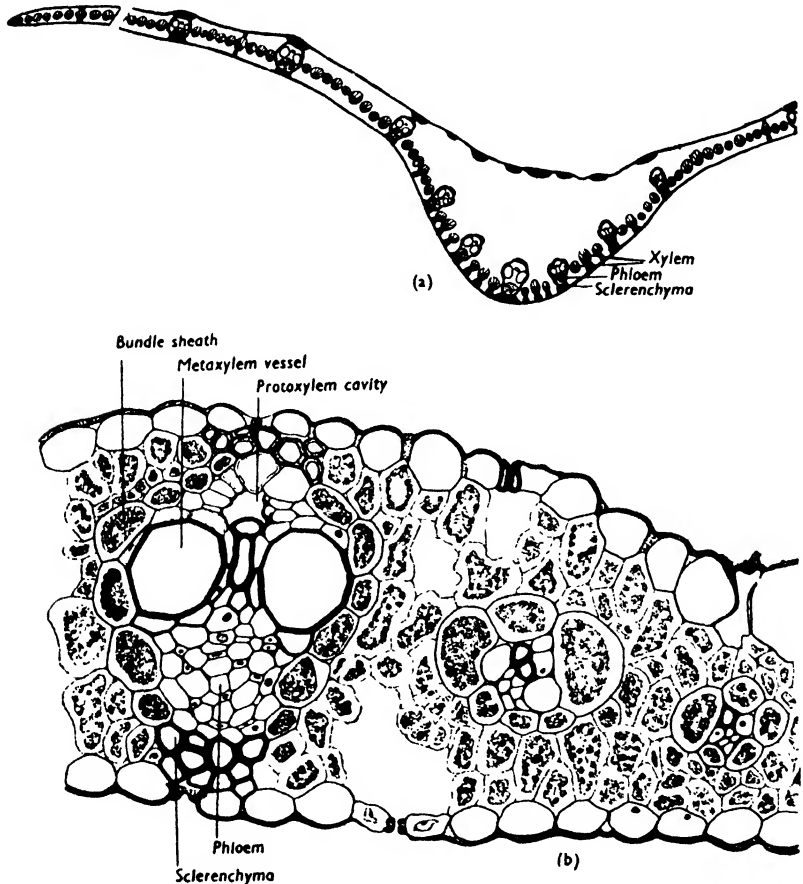


FIG. 101.—Maize, (a) plan of transverse section of the leaf to show the distribution of veins ($\times 12$), (b) part of this section under high power ($\times 120$).

the lower surface. A great part of the thickness of this region is due to the development of colourless parenchymatous tissue towards the upper adaxial surface (Fig. 101a). This region grades almost insensibly into the green lamina and is thus not a structural feature associated with one or a few large vascular

bundles, prominent on the lower side of the leaf, as in the dicotyledons.

The Internode of Maize.

Fig. 102 shows the appearance of part of an internode of maize in transverse section. The most characteristic feature of this stem is the large number of vascular bundles scattered somewhat irregularly through it. In the central region the

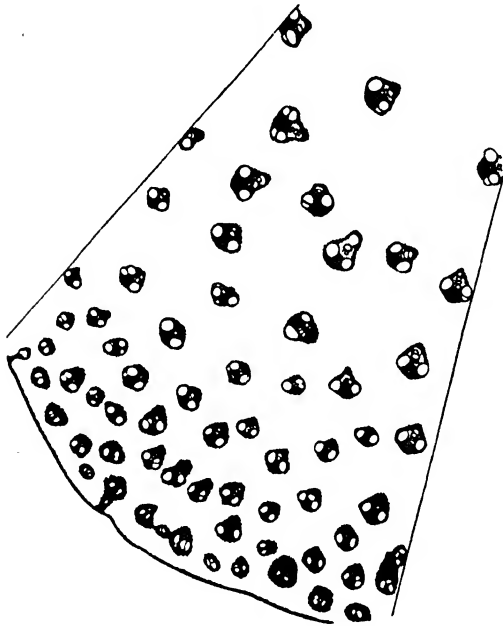


FIG. 102.—Maize, plan of part of the internode in transverse section, to show distribution of bundles ($\times 15$).

bundles are more isolated, towards the periphery they become very numerous and crowded. The peripheral bundles in particular are surrounded by an extensive sheath of sclerenchyma, which in some of the outermost bundles may become continuous with the patches of sclerenchyma developing beneath the epidermis. In still older stems this sclerenchyma development increases until the outer bundles may lie almost completely enclosed within it.

Apart from the fact that this encircling sheath of sclerenchyma varies in thickness, being least developed around the innermost bundles, where it may be represented merely by two small caps, one outside the phloem and one to the inside of the protoxylem, the bundles are all very similar in structure. Closer examination will show, however, that whilst practically all the central bundles contain protoxylem or the cavities left by disorganised protoxylem, and the two large flanking vessels that were characteristic of the large bundles of the leaf, some of the peripheral

bundles contain no obvious protoxylem and many have not the two large vessels on the flanks of the central group of xylem. There are also more peripheral bundles in which the protoxylem, though present, is not so disorganised. All these points agree in suggesting that whilst the central bundles, if part of the leaf trace system, should be continuous with the first differentiated bundles which run right through to the apex of the young leaf, some of the peripheral bundles on the other hand may be the continuations of the later formed, basipetally differentiated, smaller vascular strands in the leaf. This conclusion is confirmed by a study of leaf and stem development.

In Fig. 103 the details of a single inner bundle of the internode are represented as seen under high power. Although the oldest and innermost protoxylem is disorganised, the cavity thus formed does not disappear as in *Helianthus*. The protoxylem is surrounded by parenchyma cells as in *Helianthus*, but when

the protoxylem elements break down, the surrounding cells are not forced inwards by the pressure of the increasing tissue mass, partly because the vascular strand is completely surrounded by the bundle sheath. It is possible that this cavity may even continue to fulfil conducting functions, though this point requires further experimental exploration. On the outer face of the protoxylem are present a few lignified xylem elements, which are usually tracheids or small vessels. These lie in radial series, an order which is maintained across the phloem, and between these two tissues one or two cell rows may be present, which are the last

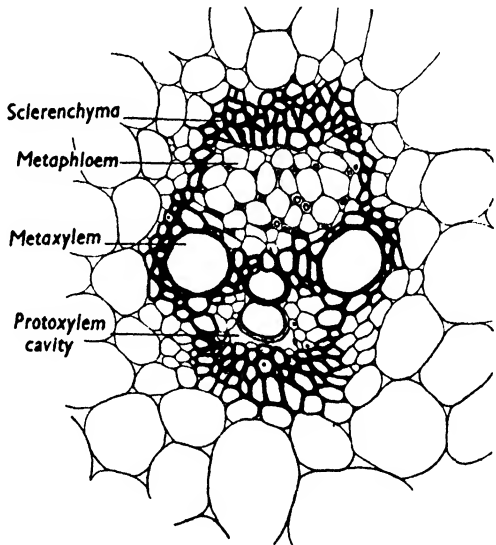


FIG. 103.—Maize, bundle from the central part of the internode in transverse section ($\times 140$).

cells differentiated from the procambium. The protophloem

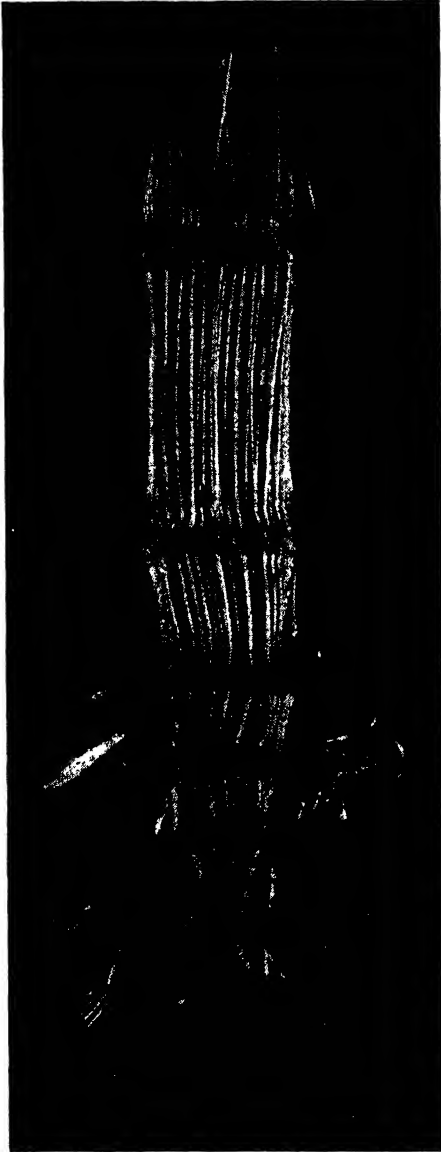


FIG. 104.—Maize, photograph of the basal part of the stem, split in half and then macerated in water ($\times 1$).

is often crushed against the sclerenchyma by the radial expansion of the later formed metaphloem; the latter tissue consists almost entirely of sieve tubes and companion cells, the regular alternation of which in each radial row gives the metaphloem a strikingly regular appearance. In the dicotyledons the continued activity of the vascular cambium was tentatively associated with two conditions existing throughout its period of activity, a supply of sap and solutes from differentiating elements, and of oxygen through the intercellular spaces along the flanks of the rays. In the monocotyledons the supplies of sap from differentiating elements are also present, but it is possible that the absence of cambial activity may be associated with the exclusion of air by the close-knit bundle sheath; such a sheath of elongated cells, without intercellular spaces, is an almost invariable characteristic of the vascular bundles of stem and leaf in this group,

teristic of the vascular bundles of stem and leaf in this group,

although the extent to which these cells may become thick-walled and lignified varies very considerably.

Throughout the length of an internode the numerous bundles take a fairly straight course, in the node they bend sharply, a number passing directly outwards into the leaf inserted at that node, others anastomosing freely and then resuming their upward course in the internode above. The complex of bundles at the node is beautifully revealed if stems of maize, after splitting longitudinally, are allowed to rot in water (Fig. 104). In such a complex type as maize it is difficult to establish any definite relation between the vascular system of the stem and the leaf trace system, but in the internode of *Alstræmeria*, where a similar scattered arrangement of vascular bundles is exhibited, it has proved possible to show that all the vascular bundles present in the internode can be connected with the leaf trace system of the two cycles of leaves immediately above.

Alstræmeria has a $\frac{3}{8}$ spiral phyllotaxis and the thirty-four or more bundles scattered throughout the cross-section of an adult internode represent the main bundles running to each of the eight leaves of the cycle immediately above, and the two laterals of about six of these leaves ($8 + 12 = 20$), together with eight more centrally placed bundles, which at higher levels take an outward course to leaves of the second cycle above, and of which they form the central strands ($20 + 8 = 28$). The additional bundles have differentiated basipetally from the leaves immediately above. The system is fairly simple to elucidate in *Alstræmeria*, as there are no complex fusions of strands at the nodes as in maize and many other monocotyledons, but none the less, even in this type, the development and interpretation of the vascular system is far too intricate to be carried further in this preliminary study.

The reader may, however, be reminded in conclusion that the course followed by many of the vascular strands in the axis of maize and *Alstræmeria*, from the central position in the axis, ultimately out into the leaf, is to be expected from the method of development of the new primordia at the apex. It is this characteristic, coupled with the large number of separate vascular strands that may join the axis as the leaf trace of one large cylindrical leaf, that tends to give to the

monocotyledon axis the apparently random "scattered" distribution of the vascular strands, which really is a much more organised system than is superficially apparent. The monocotyledon structure is thus the typical result of an axial construction that is built up from the cylindrical leaf trace systems of a series of concentric leaf insertions.

CHAPTER XXVII.

GROWTH CURVATURES. THE ROOT SYSTEM.

Direction of Growth of Shoot and Root.

In a seed the two growing regions of the embryo are already clearly indicated, the shoot system with its succession of leaves, and the conical root apex. On germination these differences in appearance are found to be correlated with characteristic differences in direction of growth. In the dark, no matter how the seed is orientated, as growth proceeds the shoot curves until the apex is pointing upwards and the root until its apex is directed downwards, these growth curvatures are described as tropisms. In this particular case the direction of curvature seems to be determined by gravity, and as a general rule the root is positively, and the shoot negatively, geotropic.

If a germinating seedling of a bean or pea with a root about half an inch long, is pinned to a cork and then kept in a moist atmosphere where its growth can continue (as, for example, in a bottle lined with moist blotting paper), it is possible to demonstrate that, in the absence of the graviational effect, both root and shoot will continue to grow in the same straight line. By means of a klinostat, the bottle in which the seedling is mounted may be kept revolving slowly (about once in twenty minutes), so that the seedling turns about its vertical axis, which is kept horizontal. Under these conditions both root and shoot continue to grow out in the horizontal direction in which they were originally pointing, but if the revolution is stopped, within a few hours the root will curve downwards and the shoot upwards. If this experiment is carried out with the bottle covered with dark paper to exclude the light, it provides evidence that the effect is due to gravity. A further point is illustrated if the root is first marked with Indian ink, with horizontal marks one millimetre apart. As

growth proceeds, the dispersion of these marks shows that the region of maximum root extension lies a short distance behind the root apex. When curvature occurs it takes place in this region of maximum extension, so that it is brought about by a modification of the normal growth processes.

That light is responsible for similar tropic curvatures of the shoot, is readily shown by placing a growing plant where it is lit from one side only. Seedlings which have been grown previously in the dark are especially sensitive, and the young shoots will bend towards quite a weak artificial light within a few hours, but plants grown in normal light alter their direction more slowly, and only in response to stronger light, such as daylight.

Shoots usually grow towards a light, or are positively phototropic, whilst the leaves they bear spread their surfaces at right angles to the light, and so long as the shoot is still growing, it is often possible to demonstrate a change in leaf position associated with alterations in the direction of the incident light. Roots, on the contrary, are far less sensitive to light, and those of many plants show no directional response at all. If seedlings of white mustard (*Sinapis alba* L.) are grown in a glass jar lighted from one side, the root will be found to turn away from the light.

Whilst general differences in tropic behaviour of shoot and root are quite definite and easily demonstrated experimentally, it must be remembered that a very wide and diverse range of phenomena is included under this head. Thus though the first root of a seedling is positively geotropic, branches of this root grow out obliquely, whilst branches of the second or higher orders may even be found to be growing slightly upwards. The geotropic behaviour of such branches will promptly alter if the main root is severed, when the tips of some of the remaining branches soon turn vertically downwards.

Similarly the first shoot of the plant turns upwards, though in the dark the apex may remain curved in the plumular hook, so well developed in alternate leaved plants (Fig. 14a). When the branches are developed, their direction from the vertical varies considerably, especially the lower, lateral branches which may grow out almost horizontally. Runners and rhizomes are extreme cases of this tendency.

These tropic responses have been so incompletely explored that it would be unwise to give too long to their discussion, but one remarkable experiment deserves brief mention, as it seems likely to be associated with a new chapter in our knowledge of these phenomena.

The coleoptile of the oat, or other grass seedling, is very sensitive to light, especially if it has previously been grown in the dark. Such a coleoptile, if only the tip is illuminated from one side, will bend towards the light, the curvature taking place in the lower, unilluminated part. The interest of this observation is that the stimulus, received in one part, induces a response by differential growth in a part at some distance from it, and in a plant, which is without a differentiated nerve system comparable with that of animals, the mechanism by which the stimulus, received at the apex, is resolved into movement in a lower growing region, obviously demands inquiry. If the tip is cut off no response to unilateral lighting is observed for a period of about two hours, but it occurred to an ingenious experimenter to stick on the severed tip again with gelatine before exposure to lateral illumination when the normal phototropic curvature was again observed. This experiment needs delicacy of technique, but it has now been repeated many times and with many variations and there is no doubt that the reception of the stimulus in one region may lead to growth responses in a region that is not directly stimulated and which may be separated from the region of reception by some medium such as gelatine. This observation has led many botanists to adopt the conception of hormones or "chemical messengers" from animal physiology, but since in the plant the effects observed are growth changes it is preferable to use the term growth substance or auxin (Gk. *auxesis* = growth). Within recent decades a very extensive literature has grown up around this aspect of plant growth, but we still have only a very incomplete understanding of the subject and it must be borne in mind that growth is a complex phenomenon which includes at least the two main phases of cell division and cell enlargement. In the elongation of the oat (*Avena*) coleoptile in these experiments the tropism results merely from differential extension of the cells on the two sides. The validity of the auxin theory for this case seems established and the evidence is good that certain growth substances are usually present in greater

concentration at the coleoptile tip, and that they move rapidly in a vertical direction down the coleoptile and affect the rate and degree of cell extension in the more basal regions where, in this monocotyledonous plant, cell extension is still proceeding. If coleoptile tips are cut off and placed on 3 per cent. agar, the auxins diffuse out and collect in the agar; small blocks of such agar can replace the normal coleoptile tip as a source of auxin and produce effects which are not produced by similar blocks of untreated 3 per cent. agar. By increasing the auxin content of the agar block above that of a normal coleoptile tip, the degree of cell elongation may be raised above the normal which suggests that in the oat seedling the auxin supply may be a limiting factor. The auxin-containing blocks produce curvature of the basal region if placed excentrically on the decapitated coleoptile, the curvature taking place *away* from the side of application of the auxin owing to greater elongation of the cells vertically below this point. In unilateral illumination a redistribution of the auxin appears to result and the curvature is *towards* the source of light.

Reliable evidence is accumulating as to the chemical constitution of three substances which occur in plants and probably serve as natural auxins and various other substances which do not occur in plants are found to have similar effects; these substances are varied in chemical formula but have certain atom groupings and features in common. It is not clear, however, whether all these substances are actually auxins themselves, or whether they owe their effect chiefly to activation of the auxins known to be present in plants. The optimum requirement of such natural or artificial auxins is so low that it seems improbable that they serve as food substances; the part they play in plant metabolism is not known, though it seems possible that they may have some effect on mobilisation of food reserves which would be in accord with the tendency to attribute to them a rôle in the co-ordination of growth processes in the plant.

The whole conception of growth substances becomes much more nebulous when carried beyond the much-studied case of the grass coleoptile. In the root, where the distribution of growth will be found to be very different, the effect of auxin is usually to retard cell extension and produce curvatures *towards* the seat of application. Besides the case of the grass seedling, in the shoots of many plants auxins exert a stimulating

effect upon the enlargement of immature, vacuolating cells such as those of pith, cortex and wound callus. In a smaller number of cases there is evidence that cell division may also be affected and particularly in quite a number of plants auxin application to stem cuttings of both dicotyledons and monocotyledons increases the production of roots. In a few instances there is a little evidence that the basipetal transmission of cambial activity and vessel differentiation from growing buds may also be linked with the auxin story. However it is dangerous at the present state of our knowledge to apply the conception of auxin control to any plant or any growth process in plants which has not been fully investigated.

The effects of such growth promoting substances obviously have possibilities of great horticultural importance. Already treatment of cuttings with auxin solutions (particularly indoleacetic acid) has been used to promote the rooting process, and it has been found possible by this means to increase root production and therefore to carry out vegetative propagation by means of stem cuttings of certain woody plants, untreated cuttings of which only root with difficulty. Spraying with auxins has also been found in some plants to stimulate the growth of the ovary of an unpollinated flower into a seedless fruit and thereby to replace the stimulation to renewed growth which is normally provided by pollination.

The Root System.

From the first stages of growth these differences of tropic response in shoot and root are associated with an entirely different manner of growth, and with striking differences in organisation. Like the shoot, the root has an apical growing region, but instead of developing a series of superficial folds, the root apex remains smooth and conical and its growth activity leads mainly to increase in length.

Growing in the dark and in soil, the root system, except in its older parts, remains white, and close observation shows that the texture of the root surface differs from that of the shoot. If a seedling bean is examined, the white shiny surface of the hypocotyl can be seen to change somewhat suddenly to a duller surface, at a zone (the collet) where the diameter of the axis also undergoes a sudden diminution.

The dull surface of the root is due to the absence of a cuticle and is frequently correlated with the outgrowth of many of the external cells into elongated root hairs. The absence of cuticle over the root surface is also suggested by its behaviour to water. If a drop of water is placed on the shoot, either on stem or leaf, the surface does not wet readily and the drop as a consequence does not spread, but on the root such a drop readily spreads into a thin film. If the seedling has been grown in sawdust or soil, the effect of this difference in surface makes itself evident, for the particles cling closely to the hairy, moist root surface, whilst they do not adhere to the shoot. It is in part this behaviour of the root system, which makes it very difficult to uproot and clean for study, that accounts for our very meagre knowledge of this system as compared with the shoot.

A useful root system for examination is that of the seedling of chickweed (*Stellaria media* Vill.), which can be found in cultivated soil at almost any season of the year, but any small seedling is suitable. The roots are carefully freed from soil by washing with a soft paint-brush and are then carefully spread out on a glass slide in a drop of water or dilute iodine, for examination with the low power of the microscope (Fig. 105). The roots are white and slender and sufficiently transparent to show some detail, particularly towards the tip and in the central core of the root, where evidently less air is dispersed in the tissues. It is readily seen that the cells are arranged in longitudinal series parallel with the long axis of the root, so that the growth in length of the root is due to the constant recurrence of transverse divisions in the growing cells. To the very outside, at the tip of the root, the cells seem less regularly arranged, they are larger and have apparently ceased to divide. In microtome sections of the apex these observations receive full confirmation, as the denser, actively dividing cells are seen to lie just within this cap of vacuolated cells. Evidently the essential feature of the growth organisation of the root apex is the way in which the cells continue to grow and divide in deeper layers within the apex, whilst the external superficial cells vacuolate and cease to grow. The cap of vacuolated cells, known as the root cap, is added to from within, as the growth of the root continues, but the outer cells wear away, especially those on the flanks of the apex, so the

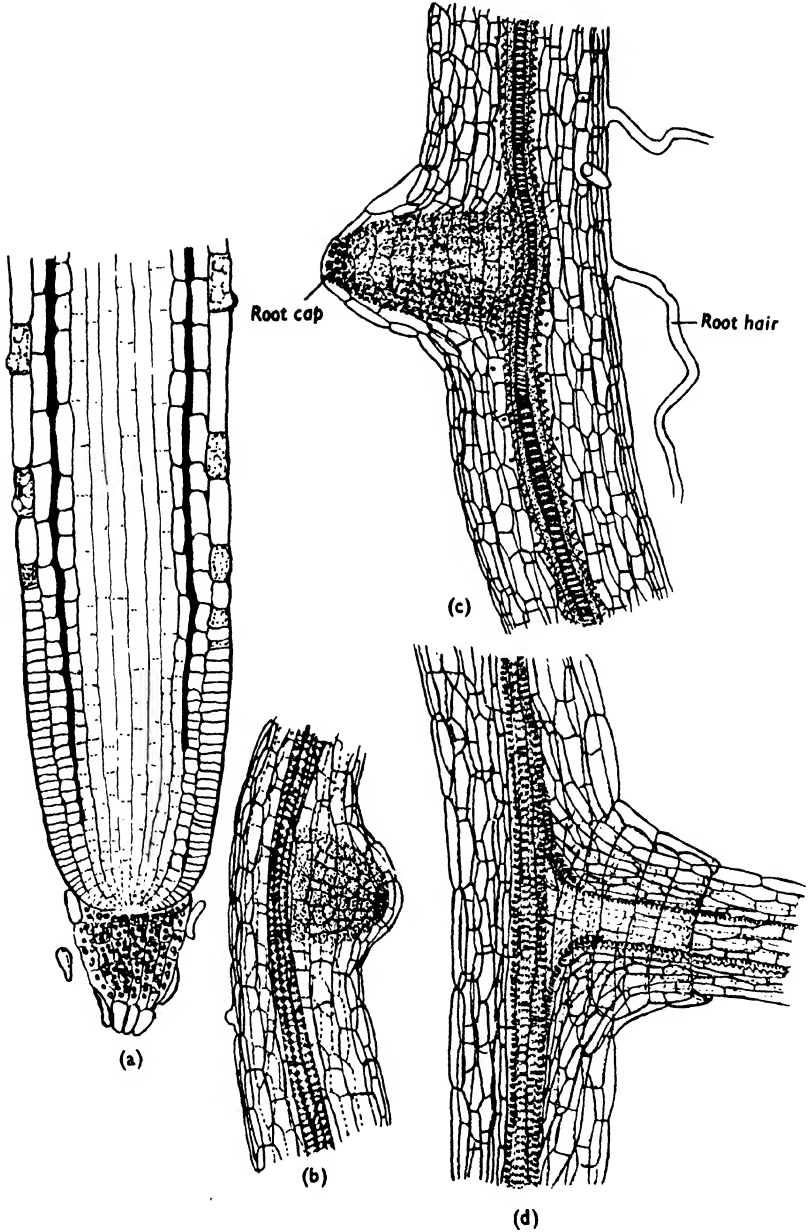


FIG. 105.—Root systems of seedlings under low power of the microscope. (a) The root apex of a grass (*Poa annua*), showing air in the cortex round the stele ($\times 150$), (b)-(d) stages in the endogenous origin of branch roots in *Veronica Chamædrys* ($\times 90$).

cap does not continue to increase in bulk. As the cells of the cap on the flanks of the apex wear away, cells of a deeper lying layer, which have been actively dividing as part of the meristem, become exposed as the superficial cell layer of the root, and this method of development of the outermost layer accounts for the absence of a cuticle over the surface of the root.

The Root Hairs.

The surface cells of the root, as they emerge from beneath the root cap, are seen to be small, with relatively dense contents and arranged in regular longitudinal rows. Farther back from the apex the cells are vacuolating and increasing in size, especially in length, more rapidly than they divide. During vacuolation, the pressure due to the water taken in by the expanding cells seems to be too great for the external walls of many of the superficial cells which, as a result, grow out as the long fine root hairs, which may exceed an inch in length, though still only a single cell (Fig. 105). The hairs are invariably injured when seedlings are removed from soil, but they may be seen very well if seeds of mustard or cress (*Lepidium sativum* L.) are germinated on pieces of flower-pot over damp sand in a moist chamber. After a few days some of the root hairs have reached their full extension so that, in a symmetrical root apex the zone of root hairs widens for a certain distance back from the tip. In older regions of the root the hairs usually collapse and disappear, though in some plants they may persist and become lignified.

The growth of root hairs is extremely rapid, in fact if a root with developing hairs is mounted under a cover-glass, it is possible to watch the extension of the hair under the microscope. The wall at the apex of the hair remains extensible for some time, and is continually being pushed out into a longer tube by the pressure of the vacuolating contents. As this takes place the contents of the cell are continually moving forward in the direction of the tip of the hair, and this flowing movement of the protoplasm is easily visible under the microscope. Just behind the tip of the hair the protoplasm is less vacuolated and usually contains the nucleus.

In the tip, the protoplasm remains in intimate contact with the extensible wall and is driven against it under pressure of the sap in the vacuole behind. In spite of the great speed of extension, the deposition of wall substance by the protoplasm is able to keep pace with it. In some cases root hairs are found which have burst at the tip, but this is probably due to injury during mounting; it frequently occurs if the hairs are examined in water.

Branching.

It is usually possible to find branches emerging from the seedling roots, but the first branch will be found a considerable distance back from the apex. They appear in acropetal order, that is in regular succession with the youngest one nearest the apex. A new branch appears at first as an almost hemispherical protuberance inside the tissues of the parent root (Fig. 105*b*); similarly it is possible to see that the older branches make connection, through the outer tissues of the parent root, with the cylindrical central core (Fig. 105*c* and *d*). In the bean seedlings examined earlier, this origin of branch roots from the inner tissues was evident, as it could be seen that the branches had split the outer tissues of the parent root as they emerged (Fig. 3).

In the dicotyledons the root system usually has a main or tap root from which branch roots emerge, and the whole root system is frequently built up by repeated branching, but in monocotyledons it is seldom possible to recognise a main root for long. In the maize seedlings a main root was present at first and this developed branches, but at later stages of growth this root was no more conspicuous than others developed from the same region of the axis, or even from nodes higher on the stem (Fig. 104). Root systems of this type are described as fibrous, as compared with the tap root systems of most dicotyledons. In some monocotyledons, such as the iris, branches are freely developed on the roots, whilst in others, as for example those of the hyacinth, no branches are ever formed. It may be remembered that many monocotyledon roots, after a period of vigorous growth, begin to die progressively from the base towards the apex, and that this is accompanied by a contraction in length of the inner

tissues and an associated throwing into folds of the outer layers.

Although tap and fibrous root systems are not invariably linked with the two groups of dicotyledons and monocotyledons, respectively, it is readily seen that these different root habits are naturally associated with the different growth habits of the shoots of these plants. In the dicotyledon we have seen that the expansion of leaves from the buds initiates cambial activity in the axis beneath, and that new tissues continue to be produced in a basipetal direction so long as more leaves are developing above. This activity must clearly be associated with the downward movement of food from the leaves, and the growth in girth of the lowest internodes must mean that some of the food has reached them. But if it is carried so far, it is very probably carried still further and made available for the continued growth of the original root system below these lowest internodes, with the result that the tap root and its branches may persist for a very long time in a tree. In monocotyledons, however, there is no continued cambial activity (Chapter XXVI), and though the primary growth and expansion of the internodes is relatively long continued, when this is completed there is little possibility of further growth in these parts or in the root system below. As leaves are borne on higher and higher nodes, additional roots are also formed in association with them. Thus there follows at the node, in succession, first the growth of the leaf, then of the axillary bud, and lastly of a series of roots, as seen for example in the common greenhouse plant *Tradescantia fluminensis* Vell. There are apparently exceptions to this behaviour, and it would be interesting to have a clear description and explanation of the relations between leaf activity and root growth in one of the tall slender palms of the tropics, in which the root system is buried in the ground at the base of the tree, whilst the growing leaves are carried aloft on a tall shaft in which there is no persistent cambial activity.

The root systems of plants are so richly branched in the soil, and their ultimate ramifications are so delicate and so intimately associated with the soil particles, that it is always a difficult and laborious task to obtain the root system of even a small plant in anything approaching entirety. In some cases this has been done by carefully washing out the soil from

a block containing a root system by means of a stream of running water, and it is a little staggering to see the total length of the root system of a single wild oat plant, grown free from competition over a period of 80 days, computed at 54 miles. The root systems of three-year-old plants of slender wheat grass, brome grass, and crested wheat grass were estimated at 9.9, 65.2, and 315.4 miles respectively.

The Root System of the Woody Perennial.

The development of the root system of the woody perennial must show an interesting sequence of changes, but it is not surprising that the story is as yet incompletely known, considering the practical difficulties in the way of its study. The roots are repeatedly branched so that branches of the first, second, third, etc., orders are formed, and the branches of each successive order usually tend to fall off in length. Along the main root there are a number of branches of the first order and some of these grow into roots of considerable length; these bear branches with less extension growth, and so on again in order, until the ultimate branches may be very short indeed. The branches of each order diverge outwards from their parent root, so that the root system spreads forward into the soil in such a way that the young vigorously growing regions of the roots of all orders are to be found in a peripheral zone, which gradually works farther and farther out through the surrounding soil. We shall see later that the zone of root just behind the apex is the most active in absorption, so that this method of growth must mean that, as the young perennial grows, its absorbing system spreads outwards through the soil and, as it does so, the richness of the branching ensures that the zone of the soil near the periphery of the root system is traversed in all directions in a most thorough way by the young branches and their covering of root hairs.

As the root system occupies in this way an ever-widening periphery, branches of still more subordinate orders are formed at the absorbing front, but within the peripheral absorbing zone the root system becomes less ramified, and it is clear that many of the branches, originally forming part of the absorbing front, have since disappeared. As in the shoot system, the extension growth of a root is completed comparatively early, and this may be followed by increase in

girth due to cambial activity, but this is vigorous only in a limited number of the branches. Others make little secondary growth, and die. Sometimes traces of the dying branches, or the scars left after their disappearance, may be seen on the persistent roots, but the exact manner of disappearance of the branches is difficult to follow since the root system during its normal development is always in the soil. The main branch system of the root thus cleans itself of a number of subordinate branches, so that the "scaffold" roots that remain are more widely spaced along the main root, and the same applies to the branches of other orders as well. The scaffold roots may grow for a very long time, and their development will obviously depend in large measure upon the suitability of the soil along their direction of growth. In time all traces of the regularity of the original root system may be lost, it may not be possible to recognise the original tap root, instead we should find a complex tracery of branching roots which ramify through the soil in all directions.

The growth in length of roots continues to a greater or less extent throughout the greater part of the year, and in a comparatively mild climate, such as that of the British Isles, growth probably stops only during the coldest months from about December to March. There is usually a vigorous production of slender root branches in the spring, and later, after a period of slower growth, another vigorous production of roots in late summer or early autumn.

The proximal portions of the root system, which thicken up considerably, undoubtedly commence and continue their radial growth each year in connection with the impetus to radial growth that passes down to the base of the tree from the leafy shoot system above. The part of the root system which adjoins the shoot is often very greatly thickened, but distally the root narrows quite quickly to about the thickness of a pencil and then, without appreciable alteration in diameter, may extend for many feet or yards before a region of repeated branching is reached, which indicates proximity to a zone of absorbing root surfaces. These long slender, whip-like roots seem to grow slowly in radial thickness almost all the year round, and it is possible that this very slow growth may be only indirectly correlated with the activities of the shoot system above.

When one considers the diverging branches of a root system, it is obviously very natural that the branches will often lie very close across each other, and, in the case of some of the main scaffold roots, some must come in contact as they thicken. The same is often true of the roots of different trees which are growing close together. As such roots continue to thicken, the parts in contact will undergo considerable chafing and pressure, and if the roots are from the same tree or the same species, their tissues often fuse together until their cambial zones become coalescent, so that with continued radial growth considerable anastomosis of the main scaffold root system takes place.

Mycorrhiza.

In many of our forest trees, as the root system becomes more fully developed, the new branches assume a very different

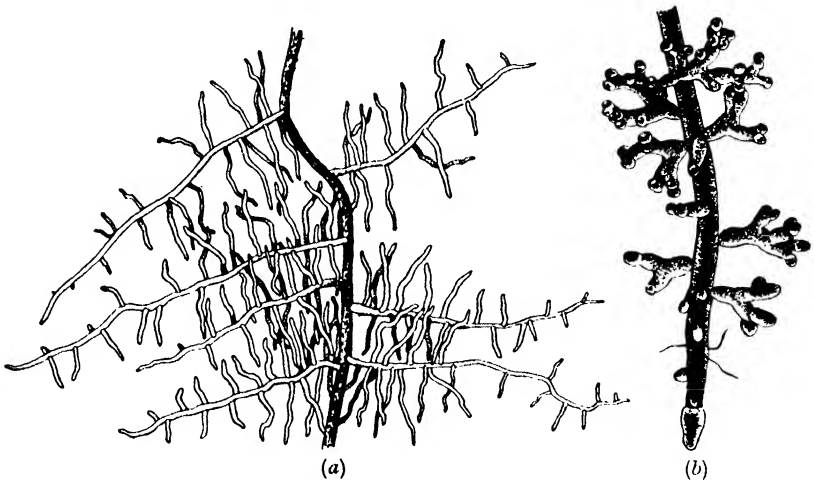


FIG. 106.—Mycorrhizal root systems in (a) birch ($\times 2$), (b) pine ($\times 4$).

appearance. They are very short, their tips soon cease to grow, whilst the region just behind appears whiter and more opaque than in normal absorbing roots. In pine the young branches undergo frequent forking, so that clusters of small rootlets are formed; in birch and many other hardwoods, though the main root of the branch still bears lateral branches, these all remain short, so that the ultimate ramifications of the mycorrhizal roots (Fig. 106), though still characteristic

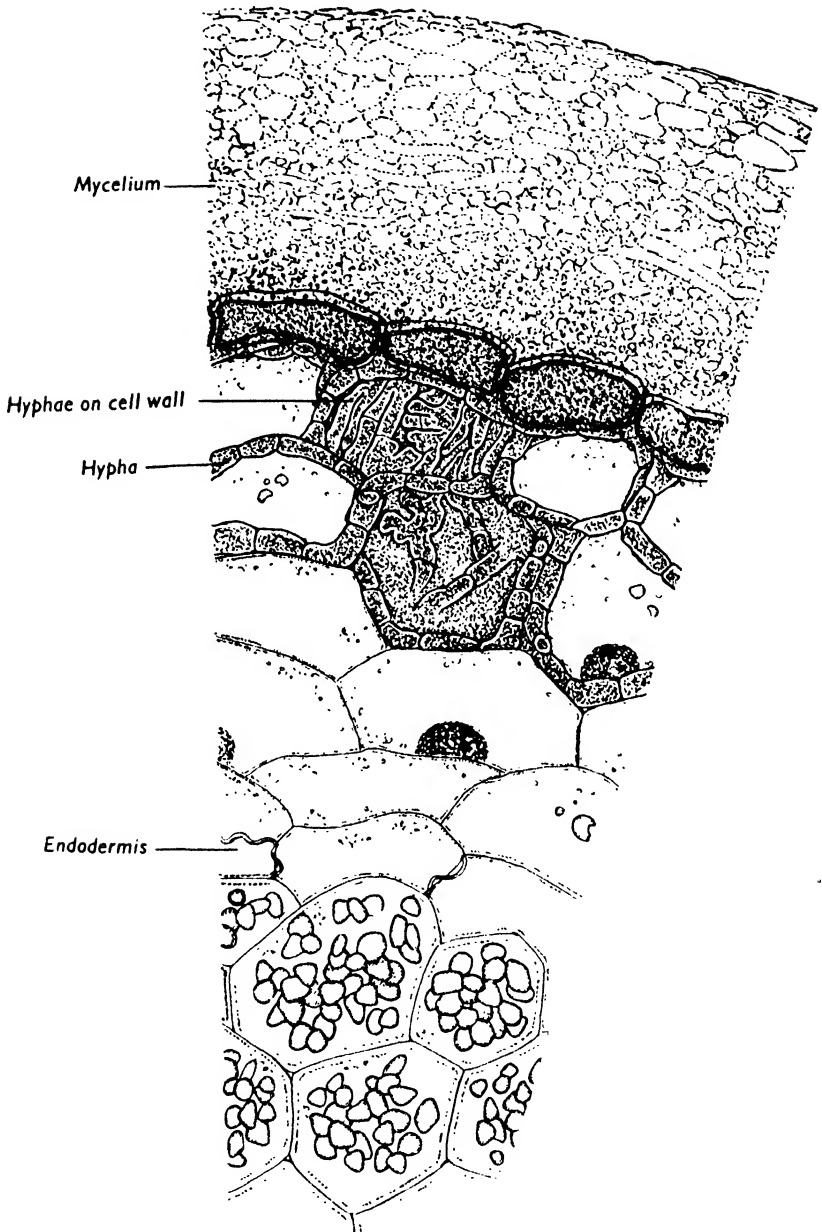


FIG. 107.—Pine, part of a mycorrhizal root in transverse section; within the endodermis starch grains are present but no fungus ($\times 650$).

of the species, are very different in appearance from comparable regions of a young tree of the same kind.

If these roots are examined under the microscope, they will be found to be covered with a close web (mycelium) of fine threads, which do not belong to the tissues of the root at all but are the colourless filaments or hyphæ of plants belonging to the group of fungi. The exact relationship of the fungal hyphæ to the root system is little understood, but we do know that the fungi have the power of absorbing nourishment from the decaying organic matter in the ground, and it has also been seen that many of the fungal hyphæ develop small outgrowths which penetrate into the living cells of the root and subsequently disappear again as though digested. Foresters have also come to the conclusion that in many cases trees grow better if their roots have formed an association of this sort with a fungus, than if they continue to grow free from it. To this phenomenon the name mycorrhiza (*mycor* = fungus, *rhiza* = root) is given. It is possible that the fungus also gains from the relationship, and in that case the development of mycorrhiza may be an example of symbiosis, the term used to describe the intimate association of two different organisms to their mutual benefit.

Fig. 107 shows the appearance of a mycorrhizal root of *Pinus* in transverse section; the close felt of threads over the surface of the root is composed of hyphæ which run mainly in the direction of the long axis of the root, so that most of them are cut across transversely and give the appearance of an outer pseudo-parenchymatous tissue. In the cortex of the root the threads run mainly between the cells, and lie especially thickly in the intercellular spaces. When the surface of the wall of a cortical cell is seen in focus, the outside of the wall is seen to be covered with a close pattern of repeatedly forking hyphæ. The central, smaller-celled part of the root is free from the fungus threads.

CHAPTER XXVIII.

THE STRUCTURE OF THE ROOT.

THE examination of the root system of seedlings as described in the last chapter, enables several of the main structural features of the root apex to be determined, as for example the position of the most meristematic region at some little distance behind the actual apex of the root, the presence of a vacuolated root cap distal to the meristem, and the regular seriation of the cells resulting from repeated transverse divisions. These features may be confirmed and examined in greater detail by the use of a microtome section (about 8μ in thickness), cut longitudinally through the root apex and suitably stained. For this purpose it is important that the section should be very near the median plane. The root apices of different plants differ only in detail, and the description that follows for the root of *Chlorophytum* may be used to elucidate the features of almost any other type that may be convenient to examine. The dense meristematic tissues form a rounded cone, covered distally by the more vacuolated root cap. In the cone the longitudinal files of meristematic cells converge to a central patch of cells at the apex, and it is evident that the files all originate from this region. It is also seen that this same patch of cells is responsible for the formation of the cap, for cells are cut off by periclinal walls towards the outside of the meristem, and this cell seriation may be followed out into the cap until it is lost owing to the rapid vacuolation. The root cap extends for some distance around the flanks of the meristematic cone, partly as the result of occasional cell divisions but chiefly through longitudinal extension of the cells. Owing to the very different manner of growth of the cap and the outermost layer of meristem just within, it is obvious that these tissues must move relatively to one another. As the result of repeated transverse divisions, the surface layer

of the meristem gradually emerges from beneath the margins of the cap, to become the piliferous layer bearing the root hairs.

In the apical meristem itself two regions stand out as particularly active in cell division and growth, the superficial layer of the cone and an inner narrower cylinder which delimits a central core. Between these there is a cortical region in which the cell contents are much less dense, and in many roots this region appears to consist of vacuolating and dividing cells, in which short files of cells, each within a common thicker wall, may be detected. When living roots, such as those of a grass, are mounted in water, the air in the spaces between the files may be followed down in the cortical region almost to the meristematic patch (Fig. 105*a*). The cells of the outermost cylinder within the root cap, more accessible to air than the files within, remain meristematic longer and continue to divide for some time after they have emerged from beneath the cap. This cell layer differs in structure, function and origin from the superficial dermatogen of the shoot, and therefore we may adopt Haberlandt's term protoderm for it. The outer cells of the central core also remain meristematic for a relatively long period, and it is possible that this may be due to the fact that air is brought so close to them by the development of the cortical air spaces so far down towards the apex. As this core is followed farther back, it will be seen that the central cells elongate and vacuolate before the two outer cylinders of cells bounding the core or stele. These two outer layers we shall meet again in the adult root as the endodermis and pericycle. Within them are all the elongated cells from which the vascular elements subsequently differentiate. In the root the central tract of parenchyma is but little developed so that the vascular structures lie near the centre.

The structure of the root may now be examined in transverse section a few millimetres back from the root apex, where most of the cells have vacuolated. The root of the creeping buttercup (*Ranunculus repens* L.) (Fig. 108) may be used for this purpose, but almost any root of a convenient size may be taken which is sufficiently young not to have undergone secondary changes. Air spaces are visible between the cells of the cortex, but immediately within the piliferous layer is a layer of cells which have vacuolated without the appear-

ance of spaces between them. These cells have remained meristematic relatively late and then, accessible to the outside air and to that in the cortical air spaces, the pectins and fats in the middle lamellæ of their walls have dried rapidly, so that when the cells vacuolated their walls have resisted the tendency to withdraw from one another at the corners. In many roots fatty substances accumulate in this layer, which is then readily recognised by the red staining of its fat-impregnated walls with Sudan III. In this condition it is

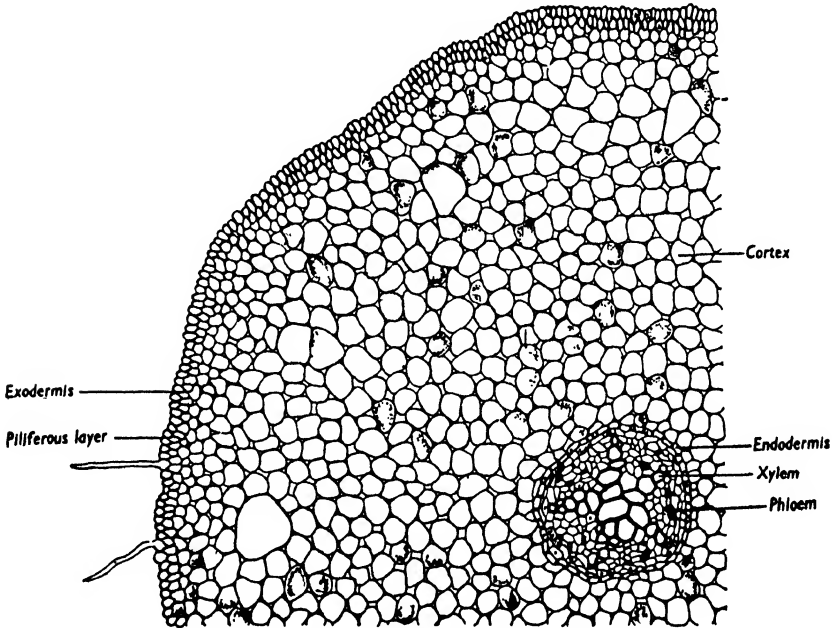


FIG. 108.—*Ranunculus repens*, transverse section of pentarch root ($\times 85$).

known as the exodermis, but even when it cannot be recognised in this manner it may still be identified as the continuous layer separating the cortical tissue, with its system of air spaces, from the piliferous layer.

To the inside the cortex is bounded by another cylinder of cells free from air spaces, which may be recognised in transverse section by a curious structural feature of the radial walls. This is a little strip of wall, known after its discoverer as the Casparian strip, which is impregnated both with fat and lignin. Though it reacts with reagents for both these

types of substances, it is most readily recognised by staining with phloroglucin followed by hydrochloric acid. In a stained hand section, the red strip on the radial wall often appears appreciably broad, but this is misleading, for in thin

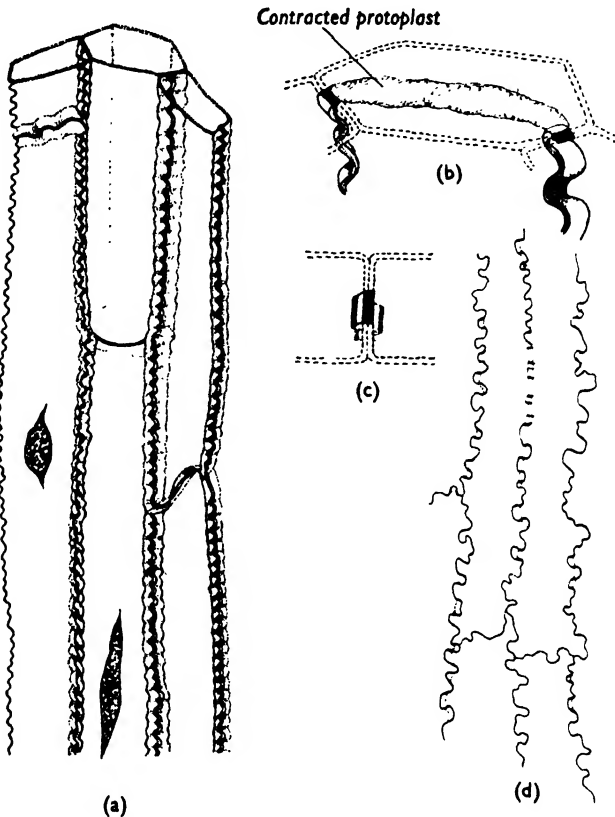


FIG. 109.—*Ranunculus repens* root, diagrams of (a) part of the endodermal cylinder showing the Casparian strip continuous round each cell ($\times 450$), (b) cell from the endodermis, in transverse section, in phloroglucin and hydrochloric acid; the contracted protoplast adheres to the Casparian strip, (c) the folded strip seen from above appears thicker than the rest of the wall, (d) part of the network of Casparian strip from a fresh section mounted in concentrated sulphuric acid.

microtomed sections it is seen actually to be a very thin portion of the wall. The explanation is that the cells always contract when cut, whilst the Casparian strip is unable to do so and is therefore thrown into zigzag folds as shown in Fig. 109a and b. As one looks down upon any but the thinnest section of such

a fold, the strip appears as broad as the full width of the undulations of the strip (Fig. 109c).

In longitudinal sections the Casparian strip is seen to be present on the transverse walls also, so that this strip is evidently continuous around the radial and transverse walls of every cell of this layer, which thus forms an unbroken cylinder through which the air spaces of the cortex fail to penetrate. This cylinder of cells is always present in roots and is known as the endodermis. The characteristic Casparian strip is always present, but it is so thin and delicate that there is no general agreement as yet as to the nature of the substances in it. It can easily be shown, however, that it is of a much more resistant nature than normal cellulose. If a section of a fresh root is mounted in strong sulphuric acid, all the tissues of the root will dissolve with the exception of the Casparian strip and, if present, the suberised and lignified exodermis. The Casparian strip is then seen as a continuous cylindrical network of folded and convoluted ribbon (Fig. 109d) lying free in the solution, and within which some remains of the xylem persist for some time.

If we visualise the nature of the endodermis from the above facts, we may regard it as a cylinder, constructed of brick-like cells, very closely set and elongated in the direction of growth of the root. The bricks are the living protoplasts enclosed in their cellulose walls, and these are so closely pressed together around the cylinder that they are firmly cemented by the middle lamella and absolutely no space is left between them. Still as cellulose walls are permeable to water and to many substances soluble in water, such substances would be able to pass across the endodermal cylinder without passing through the protoplasts, if it were not that in between the protoplasts along both the radial and transverse walls, fatty and lignifying materials have accumulated throughout the whole thickness of the adjacent walls and the middle lamella between them. This fat-impregnated and lignified Casparian strip forms a continuous framework in which the protoplasts are inserted. In sections mounted in phloroglucin and hydrochloric acid the firmness with which the protoplasmic contents of the cells adhere to the Casparian strip is usually very noticeable. The protoplasm contracts considerably under the influence of the acid and withdraws

from the tangential walls, but remains firmly attached to the radial and transverse wall, in the region of the Casparian strip so that it stretches as a band across the cell from strip to strip (Fig. 109*b*) ; visualised in the solid this would actually appear as a membrane extending right across the cell.

In the section of a root the smaller-celled region of the stele is seen to be clearly delimited from the larger-celled cortex by the endodermis, whilst within the stele the vascular tissues will be differentiating. The arrangement of the primary vascular tissues is different in the root as compared with the shoot ; in the shoot xylem and phloem both differentiated in relation to a leaf and lay upon the same radius of the stem, an arrangement described as collateral, in the root they lie on different radii, an arrangement which is termed radial. In the dicotyledon root there are usually from two to seven groups of xylem and these alternate with an equal number of groups of phloem, an arrangement termed diarch, triarch, etc., up to polyarch. The first elements of the xylem to appear are the most peripheral, whilst the subsequent differentiation proceeds towards the centre of the root, which is the reverse direction of differentiation as compared with the stem. If a section of a fresh root, stained in phloroglucin and hydrochloric acid, is squashed, the small peripheral xylem elements will be seen to be of spiral or annular types and are therefore protoxylem elements formed during the early period of elongation of the root, whilst the wider and more central elements are scalariform or pitted metaxylem (Fig. 110).

The phloem also differentiates centripetally, and the first protophloem is recognisable even earlier than the first xylem. The end walls of the narrow sieve tube elements develop into sieve plates, but as the root grows many of the first-formed sieve tubes may collapse. The arrangement of the cells of the xylem or phloem lacks definite radial seriation as they have been formed before the appearance of the cambium and are therefore primary.

Between the endodermis and the differentiating vascular elements there is always left at least one layer of living cells, the pericycle. Like the endodermis, this layer is one which remains meristematic relatively late, and as the cells are retained within the endodermis, there is little scope for their

expansion when they eventually vacuolate. If intercellular spaces appear between them, they remain small and, since they are separated from the air spaces of the cortex by the continuous endodermis, they remain free from air. This explains the transparent appearance of the stele when fresh roots are examined in water, which distinguishes it so clearly from the cortex with its dark lines of air-filled spaces.

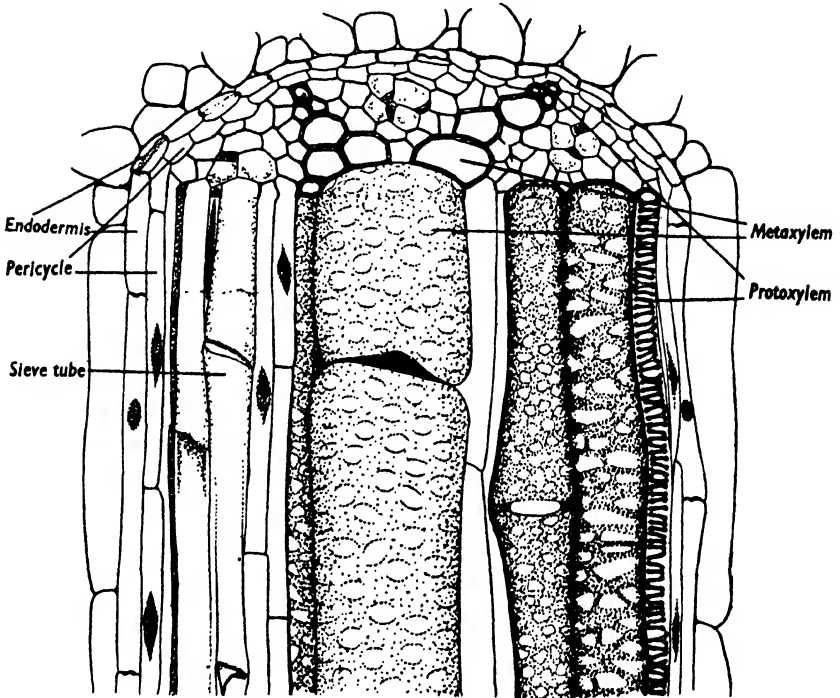


FIG. 110.—*Ranunculus repens*, the stele of the root in transverse and longitudinal section ($\times 480$).

The stele, within the endodermis, consists of vacuolating cells, some of which are differentiating into xylem at certain equidistant points distributed around the cylinder, just within the relatively meristematic pericycle. This process spreads centripetally and often the groups of xylem may meet in the centre, showing in transverse section of the root a stellate core of xylem, between the radiating arms of which, but separated from it by some parenchyma cells, lie the phloem groups. In such a dicotyledon root, if growth continues beyond

this stage of primary differentiation, it is obvious that further changes are likely to take their origin, either in the living parenchyma cells separating xylem and phloem, or in the living pericycle cells.

Secondary changes in the root are likely to be initiated in the stele. Growth depends upon food, and presumably this must be passed down to the roots from the leaves through the vascular elements. These are all found within the stele and, though the sap of xylem and phloem may be able to diffuse out of lignified xylem elements or permeable sieve tubes, it cannot readily pass out of the stele, since this is bounded by the continuous cylinder of endodermis, consisting of living protoplasts set together in a framework of fat-impregnated walls. The living protoplasts of the endodermis are as usual relatively impermeable to solutes, and especially to organic solutes such as sugars, etc., whilst the fat-impregnated Casparian strip may naturally be expected to be resistant to the movement of water or water-soluble substances. Thus the solutes from the xylem and phloem are retained within the stele, where they will be available for the continued growth of any living cells within the endodermis. Such supplies passing down the stele must, of course, be drawn upon for the growth of the root meristem which terminates the stele. We have already noted the striking difference between the root apex and the shoot apex, in that the cells which grow and divide most rapidly in the root are seated at some distance back from the actual apex, whilst the distal cells soon cease to grow. The failure of the more distal cells to grow may be due to failure of supplies of food for growth from the vascular tract; these would have to reach the distal cells either through the intervening protoplasts or through the walls and, as the protoplasts themselves are actively growing, the path may be mainly along the walls between the protoplasts. At first sight the situation here would appear to be the same as that in the shoot, where the supplies moving along such a path are evidently sufficient to keep even the most external cell layers in active growth. If, however, simple micro-chemical tests are applied, a striking difference reveals itself between the walls of shoot and root apex. In the shoot the walls of the eu-meristem readily give the cellulose reaction with iodine and either sulphuric acid or zinc chloride, but these

reactions cannot be obtained from sections of the root apex, unless these are first treated to prolonged boiling with alkali, or warming for long periods with alkaline oxidising agents such as eau de Javelle. It seems from this that the walls in the root apex are more impregnated with protein, possibly still with the living protoplasm of the cell which laid them down. Cellulose walls thus impregnated would probably still allow the movement across them of water and water-soluble solutes by diffusion, but this process could not maintain an adequate supply of solutes for growing cells over a big depth of eu-meristem, and this may be the reason why the superficial cells of the root soon cease to grow.

In any case the construction of the growing apex shows that the distal cells do soon cease to grow, and the addition of new cells from the activity of the meristem is mainly towards the inner side. We may ask, therefore, what will happen to a seedling root, when the leaves above ground no longer draw upon the food supplies of the seed but begin in their turn to send more food downwards, where it will be available for growth in the root. Since extra food apparently cannot provide for greater superficial growth, the additional activity maintained is shown in the form of more vigorous growth at various regions in the pericycle. The new growth centres are almost invariably opposite the protoxylem groups, and appear at points some little way back from the apex. These patches of new meristem soon cause a bulging of the endodermis at the points overlying them, and after a comparatively short time the meristematic patch becomes organised into a typical root apex (Fig. 105*b*). Branches on the root thus have their origin within the stele, or endogenously, unlike branch shoots which arise exogenously from superficial tissues in the axils of the leaves.

There has been much discussion as to how these branch roots make their way through the cortex of the parent root. In a seedling bean root the cortex around the point of emergence of such roots will be seen to be split, as if forced apart by the growth of the branch and, unless definite evidence of other processes is advanced, it seems reasonable to assume that these branch roots burst through both endodermis and cortex by the force of their growth. The bursting of the endodermis means the interruption, at least temporarily, of

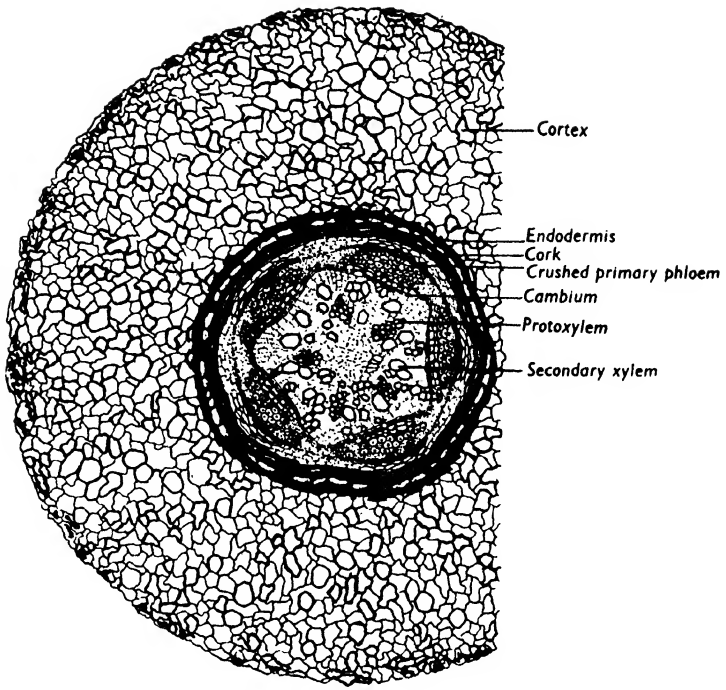
the continuity of the layer which is responsible for retaining the sap within the stele, and frequently the base of a branch root may be associated with a lump or ring of swollen cortical tissue. On this tissue, phellogen activity may give rise to cork formation or sometimes buds may appear. In plants where buds appear upon the roots, such as *Rumex Acetosella* L., or *Epilobium angustifolium* L.,* the buds are very frequently found close to the place of origin of a branch root, where evidently the leakage of sap from the stele has enabled some superficial growth to take place.

Secondary Thickening of the Root.

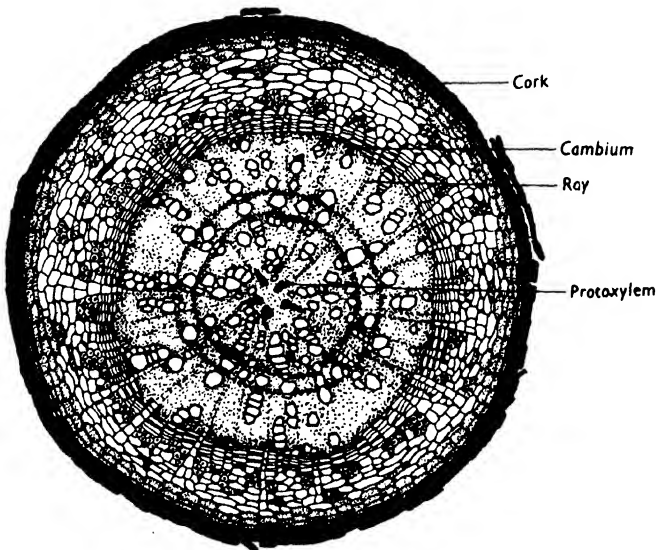
In the dicotyledon, as the shoot system develops, every adult leaf means more food supplies sent down towards the base of the shoot, and every young leaf provides an impulse to increased radial growth which starts at the leaf base and works down the stem to the lowest internode. We may expect to find, therefore, that in time cambial activity also extends to the root system, and that the downward movement of food into the main scaffold roots is associated with considerable increase in girth.

The extension growth of a root is usually completed within a few centimetres of the apex ; behind this, if the root remains healthy, growth in thickness will commence. In the elongating region there is seldom any radial seriation of the vascular tissues, so that in this respect also the root differs from the shoot, in which the tendency for the procambium to divide mainly in the tangential plane was seen first at the base of the young leaf and spread downwards through the internode as elongation proceeded. In the root all the vascular tissues formed during elongation are primary in origin, and an appreciable amount of primary metaxylem and metaphloem may differentiate before any cambial activity is noticed. The cambium always appears first as tangential divisions in the undifferentiated parenchyma, which is present just within the primary phloem. This earliest stage may be seen clearly in roots of *Ranunculus repens* L., if sections are cut well behind the region of extension, but for the later stages of secondary thickening it is necessary to cut a more woody root, such as that of lime (*Tilia vulgaris* Hayne) (Fig. 111). Lime roots are generally either tetrarch or pentarch, and the differentiation of primary metaxylem

* *Chamenerion angustifolium* (L.) Scop.



(a)



(b)

FIG. 111.—*Tilia vulgaris*, roots in transverse section after secondary thickening has commenced, (a) younger stage with the cortex still present ($\times 40$), (b) older stage in which the cortex has disappeared ($\times 45$).

usually extends practically to the centre of the root. The cambial activity starts in isolated arcs to the inside of the phloem groups. This cambium cuts off tissue to the inside that differentiates into secondary xylem, and also tissue to the outside that becomes secondary phloem, and soon forms so much new xylem that the cambium is pushed outwards and reaches a position at the same distance from the centre of the root as the first formed protoxylem elements. By this time the tendency to tangential divisions will have spread laterally, and the ring of actively growing and dividing cells is completed to the outside of the protoxylem groups by the commencement of similar divisions in the pericycle. The dividing cells outside the protoxylem may behave like the vacuolating and dividing cells of a ray, cutting off cells on either side which form regular radial rows of parenchyma. In the intervening arcs the cells are also cut off in regular radial seriation, but on differentiation some of the regularity is lost owing to the production of vessels on the inner side, and on the outer side, sieve tubes accompanied by companion cells, fibres, and parenchyma. As cambial activity proceeds, the outward thrust due to the formation of secondary tissues crushes the outer regions of the primary phloem, the position of which is usually evident in stained preparations as a more deeply stained line (Fig. 111*b*). The same force also distends the endodermis until it is finally ruptured, but before this actually happens, changes will have already occurred in the cortex and endodermis which mark the transition of the root as a whole from an absorbing to a conducting system.

In most dicotyledon roots (those of the pea family are very generally exceptions) the layer of cells beneath the piliferous layer, also free from air spaces between the radial and transverse walls, becomes impregnated, as the root ages, with fatty substances. In this condition the layer stands out clearly in sections stained in Sudan III, and is known as the exodermis. The fat-impregnation is first present in the radial and transverse walls but soon extends to the tangential walls also. It is evident that when this change has taken place, the cortical cells will no longer be able to absorb water and solutes freely from the soil, and such a region of the root is no longer part of the absorbing system of the plant. Even when an exodermis is not a conspicuous feature of the root,

somewhat similar changes usually occur in the endodermis, which have the same effect. Opposite the phloem first, each endodermal cell develops over its internal wall surface a suberin lamella, which is readily seen on staining with Sudan III, as for example in rather older parts of the roots of

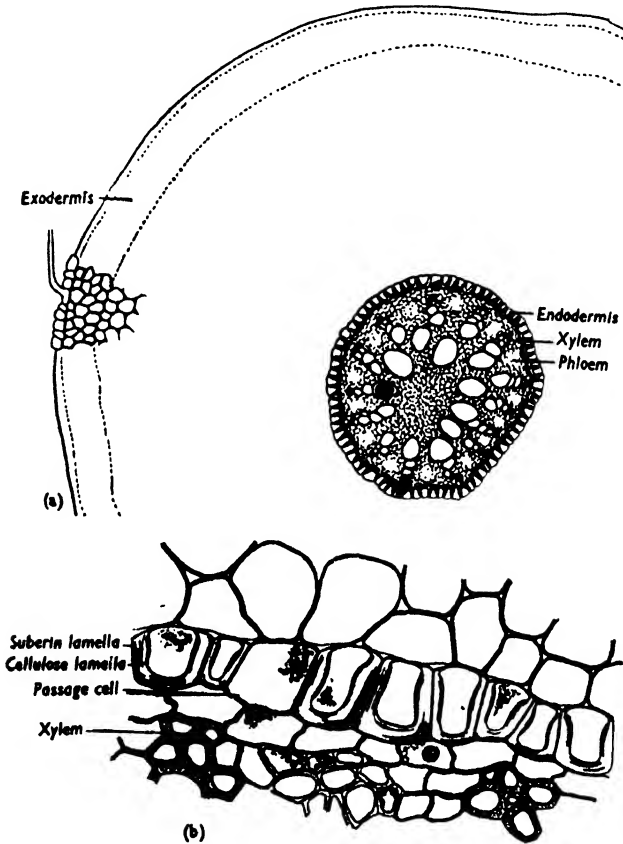


FIG. 112.—*Iris* root in transverse section, (a) showing polyarch structure of the stele ($\times 45$), (b) part of the endodermis with passage cell opposite the protoxylem ($\times 320$).

Ranunculus repens L. The fat is deposited in a layer of cellulose which is continuing to be laid down in the normal process of wall thickening, and which also continues to deposit as an inner lamella of pure cellulose, internal to the suberin lamella. The inner cellulose lamella is very thick in the endodermal cells of *Iris* roots, where the thickening is much greater on the

inner tangential walls of the endodermal cells than on the outer (Fig. 112*b*).

In *Iris* roots, certain of the endodermal cells, which lie opposite the protoxylem groups, fail to develop the suberin lamella or thickened cellulose wall. The formation of a suberin lamella will prevent much movement of water across an endodermal cell in which it is present; on the other hand, cells without this lamella, and in which the protoplasts are now growing relatively old, are probably more permeable, both to water and solutes, than the younger cells of the endodermis in the absorbing region. Such cells are spoken of as passage cells and, in the older regions of the root, they may let solutions pass across them, between stele and cortex, according to the state of pressure or tension of the sap within the stele (Chapter XXIX). If the liquid in the stele is under tension, water may be drawn in through the passage cells, but the endodermis is still an unbroken cylinder of cells and it will still be impossible for air to enter the stele except in solution. Passage cells in the endodermis are common in many monocotyledons, and possibly on this account the cortical cells outside the endodermis remain alive, even though towards the periphery an exodermis of one to several cells in depth may become suberised and lignified with prolonged exposure to the air. In dicotyledons, as for example in willow or lime roots, all the cells of the endodermis may become suberised, and in this case there is evidently no appreciable movement of food supplies across it, for the cortex soon dies and shrivels away and the older root, behind the white absorbing region, becomes thin and brown.

On the death of the cortex the endodermis becomes the outside of the root, but very often, as for example in lime, the cortex is even more completely cut off from stellar supplies by the development of cork in the pericycle, just within the endodermis. Fig. 111*a* shows the process of cork formation inside the endodermis at an early stage where the cortex, though dying, has not yet disappeared. In such a section both endodermal and cork cells will stain with Sudan III, but it will be seen that the endodermal cells do not lie on the same radial series as the cork cells, because the cork phellogen in a root arises in the pericycle, and the pericycle cells are not in radial series with those of the endodermis.

In lime, when cork development is taking place in the pericycle, cambial activity is also in progress in the stele. The resulting general expansion of the stele flattens the endodermal cells tangentially and, if long continued, finally disrupts the endodermis. Thus older roots are finally bounded externally by a layer of cork (Fig. 111*b*). In still older and thicker roots of lime, with continued expansion the outer layers of cork are ruptured and show long cracks and skin-like sheets of bark, which are peeling off, exposing fresh sheets of intact cork beneath. It is clear that the original cork layers are being ruptured and replaced by later formed layers formed by new phellogens, more deeply seated in the secondary tissues. Unlike the bark of the stem, however, the cork on the root of lime is not broken by any lenticels and the only irregularities are related to the emergence of branch roots.

Monocotyledon Root Structure.

The roots of monocotyledons have the same general structural features as those of dicotyledons, and the characteristic features of exodermis and endodermis have been illustrated by examples, drawn indifferently from either group. But the vascular elements in the roots of monocotyledons are almost invariably arranged in more numerous groups of alternating xylem and phloem than in the dicotyledons. In the latter group the number of protoxylem groups seldom exceeds seven and is more usually four or five, whilst in monocotyledons it is seldom as low as seven and is usually considerably higher (Fig. 112*a*). This may be due to the fact that monocotyledon roots spring from nodes on the stem, and are linked with numerous bundles from the leaves above. In accordance with the very short-lived nature of the cambial activity in the vascular bundles in the shoot of monocotyledons, it is found that secondary thickening in the roots only occurs in very exceptional types.

Aerial roots are very common in many of the tropical orchids and the surface layers of these roots are modified in a special manner. Several layers of cells outside the exodermis lose their contents, but develop fibrous thickenings on their walls, and also pores which communicate with the outside air. These cells soak up water very readily and in

much the same manner as a sponge, and when saturated they become so transparent that the roots show the green colour of the cortical cells beneath. Equally readily the water may be lost from these cells and replaced by air, when the roots have an opaque white appearance. As the exodermis in these roots contains many passage cells, it is probable that the water, soaked up by the dead outer cells from dew or rain, may readily be absorbed through these passage cells by the living cortical cells beneath and be passed on from them to the stele through passage cells in the endodermis. This special type of external tissue, characteristic of aerial roots, is known as velamen.

CHAPTER XXIX.

ENTRY OF WATER INTO THE ROOT. THE WATER BALANCE IN THE PLANT.

The Movement of Water into the Root.

That root systems, under favourable circumstances, will absorb water and drive it freely forward into the shoot, can be shown by cutting across the stem of a young bean plant (*Vicia Faba* or *Phaseolus multiflorus*), grown in the light, and attaching a glass tube to the stump by means of rubber tubing. A little water must be run into the tube immediately, so as to prevent the blocking of the cut surface by drying and oxidation processes. The level of the water thus added may fall at first, as some is absorbed into the plant, but as the root system continues to absorb water from the soil, liquid is driven out of the cut stump and the level of the liquid rises.

This experiment shows that a living root system, under suitable conditions, can absorb water and drive it up a glass tube, and presumably in the normal plant it may similarly be driven along the tracheæ of the xylem into the shoot. In order to understand this process, we must first examine more closely the question of water absorption by the root.

In the case of an individual cell the absorption of water takes place because both cellulose wall and protoplast surface are permeable to water, whilst within the vacuole of the protoplast a relatively concentrated sap is retained, since the solutes in the sap do not pass through the protoplasm so readily. If such a cell is placed in pure water, water is absorbed by the vacuolated protoplast owing to the osmotic pressure of the sap, but as more water enters, the swollen protoplast must expand the cellulose wall. This wall is slightly elastic, so that the cell can continue to absorb water, until the resistance of the wall to further expansion exerts a force equal to the osmotic attraction for water of the now diluted sap. Such a cell

still has an osmotic pressure but is unable to take in any more water; we express this by saying that the suction pressure¹ of the cell is nil, because its osmotic pressure is balanced by the "turgor pressure" or back pressure of the expanded wall. In any solution therefore, a living cell or a living tissue system continues to lose or gain water until, under the given conditions, its suction pressure is equal to the osmotic pressure of the external solution.

The living cells of a root system in damp soil will thus absorb water, but a different mechanism is involved in driving water up the tracheæ in the root stele and into the shoot.

Where water is plentiful in the soil and free to move, it will reach the surface of the root, whilst in drier soils root hairs emerging from the root surface will come in contact with the films of water round the soil particles. Thus at the surface of the root the cells will be in contact with the dilute soil solution and will absorb water until their suction pressure is equal to the (low) osmotic pressure of the soil solution, assuming that equilibrium is reached. Cells deeper in the root will absorb water from these cells so long as their suction pressure is greater than that of the root hairs, and in this manner all the cells of the cortex will become turgid with water absorbed originally from the soil, and similarly the endodermal cells at the surface of the stele will also become turgid, with water withdrawn from cells of the cortex. No more sap can enter these cells unless some is first withdrawn, but naturally all the living cells within the stele will tend to draw water from and through the turgid endodermal cells, until they also are fully turgid. But amongst the living cells within the stele are running the xylem tracheæ with a sap containing solutes, both sugars and salts, etc., derived from the protoplasts which were present in them as they differentiated. The liquid contents of these tracheæ are no longer retained within a semi-permeable membrane and can therefore diffuse out from them along the walls of the neighbouring cells, and thus all the living cells within the stele, including those parts of the protoplasts of the endodermal cells themselves that lie within the

¹ The suction pressure is the difference between the osmotic pressure and the turgor pressure and may be defined as "the pressure under which water enters a cell when the cell is first placed in pure water." The term is admittedly an unfortunate one and there is a tendency in the United States to replace it by "diffusion pressure deficit." The original term is retained here in view of its long usage.

Casparian strip, are bathed in this fluid diffusing from the tracheæ. The tracheal sap, better called perhaps the stelar sap as permeable sieve tubes may also contribute to its content of solutes, will withdraw water from the turgid protoplasts of all the living cells with which it is in contact, and thus increase in volume. This sap itself cannot escape from the stele, as it is retained within the endodermis by the living semipermeable protoplasts embedded in the network of the Casparian strip ; across the protoplasts the solutes in the sap cannot pass, whilst across the Casparian strip neither water nor water soluble solutes can diffuse. Thus as the volume of the stelar sap increases within the stele, it must flow upwards through the tracheal elements of the xylem, whilst the originally turgid protoplasts of the endodermis and of the living cells within, will replace the water they lose to the stelar sap, by drawing more from the cortex.

So long as the root continues to grow, the solutes in the stelar sap will be replenished by the differentiation of xylem and phloem elements, and water from the soil will continue to be drawn into the root by this osmotic system. The concentration of the stelar sap is probably less than that of the solutions generally used to demonstrate osmosis in class experiments, but by using a suitable osmometer and semipermeable membrane, it is possible to demonstrate very considerable rises in the liquid in a relatively short time, using solutions which are not of great concentrations. A suitable apparatus for satisfactory demonstration of the rise of liquid due to osmosis, is a modification of the Grainger osmometer ; this is a metal container, to the lower end of which the membrane is fixed between two rubber washers and then held firmly in position by a metal screw cap. A side arm to the metal container is an improvement which facilitates the filling with sugar solution without air being trapped. If a membrane of sufficient strength to stand the pressure is used, such as two to four thicknesses of cellophane, a 10 per cent. solution of cane sugar will show a rise of about 10 feet in a 2 mm. bore capillary tube in about three days, whilst with a 2 M solution the level may rise to over 20 feet in about ten days. In the root, the surface of the semipermeable membrane is represented by the area of the endodermal cylinder, and it is probable that the concentration of the stelar sap is sufficient to bring about a relatively rapid rise of the liquid in the narrow tracheæ.

Considering this mechanism in the root, it is not surprising to find that the stumps of decapitated shoots often bleed, in fact it is perhaps more a matter for inquiry that in very many cases such stumps do not bleed at all, if cut at any season of the year, but instead absorb water when it is supplied to the cut surface. This effect is, however, intelligible when it is realised that evaporation from the leafy shoot will often draw upon the water supply in the plant, more quickly than it can be replenished by root absorption. In the spring, for a brief period, many decapitated shoots will show exudation of liquid due to root pressure, but at other times of the year, and with some plants at all times, if the stem of the plant is severed when immersed under a coloured fluid, such as a watery solution of red ink, and then immediately withdrawn from the fluid, subsequent examination of the plant will show that the dye has entered the tracheal elements, often for a very considerable distance. This must indicate that the tracheal elements in the plant must have been under tension, though the nature of the tracheal contents under these conditions is still a subject of debate. If the experiment is carried out with any leafy herbaceous plant in summer, the ink, when its course is followed by repeatedly cutting across the plant and examining the cut end with a lens, will often be found to have penetrated upwards and downwards through the tracheal system into the leafy region and into the root system, so that a large percentage of the total length of the tracheal system has been penetrated by the fluid.

In a tree during summer the rate of removal of water from the old wood, through the differentiating xylem to the leafy shoots (Chapter XXV), certainly cannot keep pace with the rate at which it is lost by transpiration. All sources of supply together fail to overtake the rate of evaporation and, from May onwards, more and more of the vessels in the newly differentiated wood, in which the files of living protoplasts have now disappeared, will be found to inject with great rapidity if they are cut open under a coloured liquid such as Indian ink. This injection is evidence that the pressure in the injecting vessels is below that of the atmosphere, and at the same time raises problems that are far from solved as to the method by which sap rises in the tree.

Trees may be 350 feet high, and from the height that the barometric column of mercury is maintained, we know that the pressure of the atmosphere can only sustain a water column of some 35 feet, so that ten atmospheres would be needed to support a column the height of such a tree, and a still greater force would be needed to drive liquid into the leaves at this height. And now we find that the contents of the wood vessels, through which the supply comes, are actually under tension, save for the youngest differentiating vessels on the outside of the wood. If the liquid contents of the wood elements are to be *pulled* up to the leaf, then the greatest tension must exist in the vein systems of the leaf, and this raises a difficult structural problem. Pitted xylem vessels with their lignified, thickened walls, in which only small, thin pit membranes are interspersed, are admirably constructed to withstand tensions, but if these tensions are transmitted from the leaves, they must also be withstood by the walls of the spiral xylem elements, that compose most of the xylem in the veins in the leaf, and it may be difficult for this tension to be supported by the areas of thin primary wall in such elements.

When the vessels are cut open under Indian ink, the great velocity with which the injecting fluid enters, raises further difficulties. Water is so inextensible, that if the vessels were originally full of sap, a nearly equal volume of liquid must move with equal rapidity out of the vessels upon injection, as their volume changes very little. This rapidity of movement means either extremely high liquid tensions and an astonishingly rapid readjustment of the distribution of the liquid in the leaf above, of which there is no evidence, or alternatively that the vessel, when injected, contained only water vapour and air at a low pressure. Either alternative raises difficulties, and we can only say at present that the problem of the ascent of sap in the tree, and indeed of water in the plant, raises a number of most attractive problems that yet await solution. One further observation may be made from such injection experiments. The Indian ink contains a very fine suspension of carbon particles, which will not pass across the walls of the vessels and, from the distance to which the injection moves, the continuous cavity of such a vessel must be very long in the trunks of trees with ring porous wood. The injections have been traced for more than 20 feet in trees of elm, oak, and ash ;

in diffuse porous woods the vessels seem usually to have a length of only a few feet, or even a few inches.

That the xylem vessels sometimes contain water vapour, probably holds in the case of the herbaceous plant also, and the presence of water vapour in many tracheal elements, after evaporation has been in excess of absorption for some time, would explain why the decapitated shoots of such plants absorb water instead of releasing it. After complete injection the tracheal system in the severed stump might once more be full of water, but whether it would now begin to force water out of the cut end again would depend upon whether the root system was still growing. It is not surprising therefore, that in many plants the severed root systems have not been observed to demonstrate this phenomenon of bleeding, which is usually spoken of as root pressure.

The Water Balance in the Plant.

The water content of the plant will obviously fluctuate, as the result of the varying balance between rate of water absorption by the root system and evaporation from the leafy shoot system. In the individual living cells, where most of the water is retained within the vacuoles of the protoplasts, the fluctuation may not be so great. When the water supply is abundant the cells will be fully turgid, but when evaporation exceeds absorption the water will only be withdrawn slowly because, as the cell diminishes in volume, the osmotic pressure of the cell contents will no longer be balanced by the pressure of the expanded wall, and the water will be retained in the cell by the suction pressure. Since the cell wall is only very slightly elastic, as the volume of the cell decreases the suction pressure increases very rapidly and, in the unextended cell, is equal to the osmotic pressure of the cell contents. Occasionally the water content of the living cells falls still further, when the volume of the cells continues to decrease, the unextended walls being drawn inwards as the protoplast diminishes in volume. In this condition parenchymatous tissues appear soft and collapsed and shoots in this state are described as wilted (Chapter XXI). Usually the check to the rate of evaporation, that follows naturally from the fact that the stomata close and the cells hold their water with an increasing suction pressure, so reduces the rate of water loss that water absorption at night,

or under moister conditions, restores the water balance before the living tissues wilt. It is evident, however, that the contents of the tracheal system must fluctuate considerably during the process. In large woody systems, such as tree trunks, special methods have shown very rapid changes in volume of the wood, and these suggest that such fluctuations in water content are occurring.

It will be remembered that in the growing regions of the plant, the continued growth and division of the cells depended upon the supply of water and solutes. The fluctuations in the contents of the tracheal system are undoubtedly correlated with fluctuations in the rate of extension growth. Observations and measurements will often show, even under English conditions, that growth takes place more rapidly at night, when the water supply overtakes water loss by evaporation. However, the method of development of the tracheal system in the shoot, where the xylem differentiates as a number of isolated systems, enables growth to proceed at the shoot apex when the tracheal systems in connection with the adult foliage are in a condition of tension. Thus a *Pelargonium* plant will continue to unfold new leaves when in dry soil and when all adult leaves are withering. A similar phenomenon is shown in the spring of every year in woody shoots where, if the surface of the newly forming wood is exposed by peeling off the bark and then an incision is made under a drop of Indian ink, the ink will be found to rush into deeper-lying and older elements of the wood, which are in a state of tension, whilst it does not enter the more superficial elements which have just differentiated and are full of sap. The younger elements are part of the continuous tracheal systems which are prolonged as the files of vessels which run into growing leaves; they have evidently withdrawn water from the older and deeper-lying elements, which are in similar continuity with older leaves which have ceased to grow, or even with scars of fallen leaves. This simple experiment shows that the youngest, living sap wood plays a predominant rôle in the movement of water into growing leaves, and we should have a very inadequate picture of the functions of the wood of the stem, if we regarded it simply as a system of pipes along which flow is possible when water is driven in from the roots below or sucked out into the leaves above. We must regard the old wood rather as a re-

reservoir in which water is stored in tracheal elements when supply is in excess of demand ; upon this store the osmotic systems of the newly differentiating wood are able to draw. The water supplies are in this way rendered available for the growing leaves with which the new vessels are in comparatively ready communication.

The whole mechanism by which water is moved through the woody system is probably very complicated, and there is as yet no general agreement amongst botanists about the processes by which water is raised to great heights in tall trees, but at least it is true that during the winter months the wood of the tree is filling up with water. Then during the months of active growth, this water supply is drawn upon for the growth of the new crop of shoots, and when these are fully expanded, evaporation from the leafy crown reduces the water supply throughout the wood to a minimum value again.

In this section the question of water balance has been discussed especially with reference to the tree, as it is here that the problem is present in most acute form, but herbaceous plants similarly develop tensions and the problem may perhaps profitably be summed up in general form. As water is lost from the more superficial cells of the leaf by evaporation, their increased suction pressure leads to withdrawal of water from more deeply seated cells and eventually from the tracheæ ; the latter will consequently develop tensions. The suction pressures of the living cells and the tensions of the xylem systems will produce a condition in the tissues which will be transmitted downwards from the evaporating leaves into the shoot. When such a condition reaches the root system, it will itself be effective in increasing water absorption. Tensions in the xylem of the root lead to loss of water to the tracheæ from other stelar cells and these in their turn will, by virtue of their increased suction pressure, draw water across the endodermis and cortex—in part along the walls and in part through the cells. Since this tension may be considerably higher than the osmotic pressure of actively growing roots which exhibit root pressure, a greater proportion of the water in the soil is made available to the plant. This water intake which has its seat in the tensions developed in the shoot is not dependent upon an actively growing root system and can in fact continue for a time after death of the root system and is therefore sometimes distinguished as a passive mechanism.

Since it is only for a short time under favourable conditions that root pressure is exhibited, it seems probable that in most plants the greater proportion of the water owes its absorption to the conditions initiated in the transpiring shoot.

Amongst the points that have been raised in connection with water supply to the shoot, it is clear that the suction pressures of living cells and the tensions set up in the dead tracheal systems are both involved. There is at present little evidence as to which of these is the more important in raising water to the leafy regions of a plant when transpiration exceeds absorption, but this brief discussion of a very complicated problem may suffice to show the activity of the root in absorbing water in its proper perspective, as one contributing factor in the balance of processes which determine the water content of the plant.

CHAPTER XXX.

THE ENTRY OF INORGANIC SOLUTES. THEIR RÔLE AND THEIR SUPPLY TO THE PLANT.

The Movement of Inorganic Solutes into the Root.

The entry of water into the root is controlled by osmosis. The exhibition of osmosis is dependent upon the presence in the system of a membrane, which will allow the passage of the solvent freely across it, but not of the dissolved solutes. But this semi-permeability of the protoplasm, a necessary part of the machinery drawing water into the root would, if complete, prevent the entry into the root of the salts from the soil solution. That protoplasm is relatively impermeable even to inorganic solutes, is readily shown if living cells are immersed in solutions of such soluble salts as potassium nitrate, sodium chloride, etc. If the external concentration is stronger than the concentration of the solutes in the sap of the cell, water is withdrawn across the protoplast and this retracts from the surface of the cellulose wall, as the external solution readily penetrates the permeable wall and follows the protoplast surface (p. 185). But this impermeability is only relative, and the phenomenon of recovery from plasmolysis, which usually follows if the living cells are left in such solutions and remain healthy, suggests, though it does not prove, that solutes from the external solution are slowly entering the protoplast, so that the internal osmotic concentration is rising. More conclusive evidence on this subject, however, has been provided by recent studies upon the concentration of salts in the solution external to the cells of a living alga, *Nitella*, as compared with the concentration of the same salts in the vacuoles of the cells. These experiments have shown conclusively that salts slowly enter the protoplast and also that their entry is not simply determined by diffusion according to the concentration gradient. Thus the ions

potassium, sodium, chlorine, etc., accumulate in relatively high concentrations in living cells, whilst sulphates do not, and in either case the relative internal concentrations show no direct relation to the relative external concentrations in which they were supplied to the plant.

This entry of salts can be reconciled with the behaviour of the living protoplasm as a semipermeable system contributing to the entry of water by osmosis. The semipermeability is evidently incomplete, and more effective towards the larger organic molecules such as sugars. As a consequence a difference of concentration is long maintained, but the salts, more exactly the ions into which they are dissociated in solution, enter the protoplast *very slowly* so that, at least so long as the cell is growing, it is slowly accumulating salts. Very probably the protoplasmic surface behaves differently at different times and in different regions. Some such conclusion seems forced upon us by the fact that even the large molecules of such substances as cane sugar must presumably enter and leave cells with considerable rapidity at times, to judge by the rate at which carbohydrates move from adult leaf to stem, and from cotyledons to growing points. One difficulty in this connection is that cells plasmolysed in cane sugar solutions seldom show any sign of recovery from the state of plasmolysis.

The internal accumulation of salts depends upon the health and vigour of the cells and is very active in growing cells. Old cells that have ceased to grow begin to release salts again; cells in water in which the oxygen supply is deficient, or the carbon dioxide accumulation excessive, behave similarly. It should be emphasised that this accumulation of salts within the plant takes place, even when the salt is simply accumulating in the sap. The ions of a salt probably enter independently, as their relative concentrations, within and without, may be quite different; naturally if any ion is withdrawn from solution in the synthetic processes associated with growth, the tendency for this ion to continue to enter may be accentuated. Since the process of salt accumulation in the living cell is not simply the result of diffusion, and the ions may be entering against the gradient of concentration, their inward movement under these conditions involves the expenditure of energy.

The absorbing region of the root is built up of living and

growing cells, so that these observations upon algal cells would point to the probability that cells of the root similarly accumulate ions, which are withdrawn from the dilute soil solution. Of recent years direct experimental evidence has been supplied that such an accumulation does take place in the absorbing region of the root. As in the case of water movement, the difficulty arises that the accumulation of ions within living cells, does not explain their movement upwards from the root, in the contents of the dead tracheæ of the xylem. Experiment shows, however, that the salts accumulated in the absorbing region can move relatively rapidly into the living cells, in parts of the root not in contact with the solution from which the ions are derived. The contents of the tracheæ were originally derived from the protoplasts of the cells from which the tracheæ were differentiated, and this source may be responsible for some at least of the salt content of the liquid moving upwards from the root.

The upward movement of salts in the plant is an almost unexplored field, but if the same type of physiological process, controls the movement, they will be found to accumulate in growing rather than in adult tissues. On the whole this deduction is again in accordance with observed facts, as growing tissues are known to have a high content of salts.

Inorganic Salts necessary to Plants.

In the accumulation of salts by algal cells, we cannot see that the extent to which particular ions accumulate has any relation to their usefulness. Thus nitrates accumulate relatively rapidly, and are obviously very important to the plant, which must use nitrogen in the construction of proteins, etc., but chlorides, which also accumulate relatively rapidly and to high concentrations, are not known to fulfil any essential functions in the cells. It would be unwise, therefore, to make a complete analysis of the ash of a plant, and to conclude that the elements found are all essential, or that their relative abundance throws any light upon their relative usefulness. In order to obtain some information as to the different inorganic substances that are necessary to the plant, the problem must be attacked by some other method. If a seedling plant is placed with its root system in a soil solution, in many cases it will grow

healthily, but if the solution is replaced by pure water, the plant soon fails. We may try, therefore, to determine the minimum requirements of a plant by growing it in water culture, and finding out what substances must be supplied in solution in the water culture in order to maintain healthy growth.

Suitable species of plants can be grown in water culture until they flower and fruit, but the method needs care and, where accurate conclusions are to be drawn, the technique of water culture requires considerable development. The soil solution is normally very dilute and the concentration of salts in the culture solution needs to be very dilute also. If this is the case, then a small volume of solution will contain a very small amount of any given salt so that, for healthy and continued growth, the volume of liquid should be relatively large or, better still, the supply should be continually renewed by circulation. Circulation also makes it easier to keep the culture liquid well supplied with oxygen, whilst the carbon dioxide accumulating from the growing root system is continually removed. But in practice such elaborations are not always possible and, in ordinary class work, all that can be done is to use reasonably large bottles and to change the solution at intervals and also, if a glass tube is passed well into the solution through the cork, air can be blown in through this tube at frequent intervals to keep the solution aerated. The plant itself is usually grown to seedling stage in sand and is then transferred to the bottle of culture solution, in which it is held in position, by firm but gentle packing, through a groove in a split cork. It is important to keep the packing material dry, or there is considerable danger of losing the seedling by a disease known as "damping off."

With simple precautions of this kind it is possible, as a class exercise, to show that the normal seedling grows well in a relatively simple solution, and that if one or other of the elements provided by this solution is omitted, then growth is not so satisfactory. The following formula is suggested for the full culture solution, and others may be devised in which in each case a single element is omitted, whilst approximately the same total concentration of the other ions is retained by the substitution of an alternative salt.

To one litre of distilled water :

Potassium nitrate (KNO_3)	. . .	1.0 gram.
Sodium chloride (NaCl)	. . .	0.25 "
Calcium sulphate (CaSO_4)	. . .	0.5 "
Magnesium sulphate (MgSO_4)	. . .	0.5 "
Calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$)	. . .	0.5 "

with a trace of ferric chloride.

From experiments of this type it is possible to compile a list of the elements which must be supplied in appreciable quantity if normal growth is to be maintained. Much more critical experiments would need to be carried out before it could be asserted whether other elements were not essential in very small quantities, and it is always possible that certain species of plants may require particular elements in addition, which are not required by all plants. The general experience now available from water-culture experiments leaves no doubt that, at least, the seven elements, calcium, potassium, magnesium, iron, nitrogen, sulphur, and phosphorus, must be available and, except in the case of iron, in relatively considerable amounts.

It is difficult, however, when such a complicated process as growth is involved, to draw conclusions as to the specific significance of an element, from the observed consequences that follow upon its absence. Of nitrogen, phosphorus, and sulphur (supplied as nitrates, phosphates, and sulphates) it can be said at once that these are all closely connected with protein metabolism, because they are found either in proteins or in the complex derivatives of these compounds that are invariably present in the living cell. It is indeed more surprising, with such compounds, that such relatively small amounts maintain healthy growth. With phosphorus, as also with potassium, it would seem that the young plant often absorbs enough in a short period of growth to last it through life, if no more should be available, but we must remember that substances, if utilised in quantity by growing cells, may be released again from them as they age, and so become available for transfer to another actively growing region. Certainly phosphorus and potassium are more abundant in the ash obtained from the more actively growing tissues of the plant. Of potassium it can also be said that a plentiful supply seems to aid vigorous root production and the conservation of moisture

in the plant, whilst calcium on the contrary, if present in relatively considerable amount, seems to reduce water absorption and to have a tendency to increase water loss by evaporation. Calcium is usually found as the calcium pectate of the middle lamella and as the crystals of calcium oxalate or carbonate, which frequently occur in the older tissues, so that calcium, unlike potassium, is found to a greater extent in adult tissues. Crystals of calcium oxalate may occur as the needle-like raphides or as more spherical masses of crystals, sphaeraphides, and these may be distinguished, by their insolubility in dilute acetic acid, from the similar, usually amorphous, accumulations of calcium carbonate.

Magnesium must be an essential element in the plant since it enters into the composition of chlorophyll, though it doubtless has other rôles to play in the plant as well. On the other hand, though iron does not enter into the composition of the chlorophyll molecule, yet a characteristic indication of its absence is the development of white "chlorotic" foliage, which will turn green in many cases if sprayed with a dilute solution of an iron salt. Iron is known to enter into the composition of the red pigment of blood, hæmoglobin, which has a chemical constitution very similar to that of chlorophyll, but even in this case, iron as a tonic seems sometimes to lead to more formation of hæmoglobin, than can readily be explained simply on the basis that the minute amount of iron added to the system is utilised to enter into the constitution of new molecules. It seems therefore that the iron may have an effect upon the construction of the complex organic nucleus, common to both chlorophyll and hæmoglobin, and with which, in the plant magnesium is combined, in the animal, iron. Certainly iron is very often present in the plant cell in complex organic combinations, in which it will not give any of the usual colour tests for the inorganic ferrous or ferric ions. Iron atoms have a power of combining with varying numbers of oxygen atoms, and this property has not been lost by the iron present in these organic combinations, which may therefore be active in the complex oxidation processes that are proceeding in the plant.

Manganese is another element, which, like iron, forms several oxides and, as a consequence, has oxidising properties under suitable conditions, and manganese has often been found

associated with the oxidases (Chapter XX) extracted from plant tissues. Manganese is not one of the elements found essential for normal plant growth in the usual type of water culture experiment, but only a very minute amount would be necessary to play the part of an element essential in the construction of an organic catalyst and, unless the chemicals used in the experiment are very pure, sufficient manganese would probably have been added as an impurity. Indeed, experiment has shown that deficient growth, even on natural soils, may in some cases be remedied by the addition of more manganese, and the evidence certainly suggests that, for some plants at least, small quantities of manganese are essential. Similarly, very careful experiments have shown that minute quantities of boron are essential for healthy growth of broad beans and tomatoes, and certain soils in California have been under investigation where poor plant growth has been traced, in some cases to deficiencies of boron, in others to an excess. Zinc and copper are probably essential in minute traces and with improvements in our methods it seems likely that some other elements, which normally occur in plant ash, may also prove to be essential trace elements.

An outstanding feature of the usual culture solutions is that, though sodium chloride is sometimes included, they frequently omit three of the elements present in the soil in greatest quantity, silicon, sodium, and chlorine. The presence of these substances almost certainly contributes to normal healthy growth, but none of them fall into the category of substances, the absence of which, in appreciable quantity, leads rapidly to deficient growth. Silicon, in fact, seems to favour vigorous nitrogen metabolism, and in the flowering stems of some grasses silicates may accumulate in such quantity that the skin may be cut when grasping them. This observation led at one time to the idea that the "lodging" of cereal stems, which makes a crop so difficult to reap, might be prevented by the addition of more silicate to the soil. Experiment has shown, however, that the stem cannot be strengthened in this manner. On the contrary stem strength is a hereditary quality which may be profoundly influenced by the degree to which light reaches the stem during development; when light is excluded by crowding, the stems are much weaker and more inclined to lodge.

No very definite rôle can be assigned to sodium and chlorine, the two elements which combine to make common salt, but, in the ordinary way, when water culture experiments are carried out in glass bottles, without special precautions as to the purity of the chemicals, traces of sodium and chlorine will be available for the plant and it appears that sodium is able, to a limited extent, to replace potassium in the economy of the plant.

Altogether it is not surprising, in view of the complexity of the metabolism of plants and of the medium in which they normally grow, that so far our experience of water cultures has only given us a provisional list of substances which are essential in appreciable quantity for normal growth, and a tentative list of other elements which may be required in exceedingly minute quantities.

As these substances are not always present in the soil in inexhaustible amount, continued cultivation on the same plot of ground soon raises the problem of supply. The harvesting of each crop of plants will remove with it the elements which have been accumulated in the plants at the time of their removal. This raises at once the practical problem of manuring.

Cultivated and Uncultivated Land.

So long as ground is uncultivated, so that plants die and decay in the place where they have grown, the surface layers of soil may become gradually richer in substances necessary for plant growth. The root systems accumulate such substances from the deeper layers of soil and, when the plants die, their remains are left at the surface.

On the other hand, under cultivation, not only are the crop plants removed when they are best developed and contain the maximum quantities of salts removed from the soil, but other plants, "weeds," are kept down as much as possible, so that decaying vegetable matter does not accumulate to enrich the soil. When organic matter (humus) is thus deficient, this also has the effect of decreasing the numbers of minute micro-organisms, whose activities enrich the soil, especially as regards nitrogen; these organisms require a supply of organic substances as a source of energy for their activities.

In order to postpone the time when manures must be

stubble is ploughed into the soil, the nitrogenous supply is increased.

One naturally inquires whether micro-organisms with similar properties cannot be cultivated on other plants, but so far such attempts have met with no success and, apart from a few isolated cases, such as *Alnus*, which also has root nodules, they seem to be almost restricted to roots of the pea family (a root system which is usually free from a suberised exodermis, a coincidence which may have some significance).

Micro-organisms which can fix atmospheric nitrogen may also be found in the soil, but they need sugars or other carbohydrate supplies so that they do not flourish unless organic matter is present in the soil. The encouragement of such organisms is one of the advantages to be gained by the practice of green manuring, when a crop, such as mustard, is grown on the ground and then ploughed in.

Future agricultural practice is likely to see more and more attention paid to the activities of the natural flora of the soil. Though most of the soil organisms are small, like the bacteria, their numbers make up for their minute size; the bacterial population of a soil may easily number from 20 to 40 millions per gram of soil, so that the result of their activity upon the food substances available for the plant may be very important. One example of this is seen in the profound effect produced by partial sterilisation of soil in horticultural practice. This practice may well be justified by the fact that heating the soil to some 70° C. reduces the numbers of disease-producing organisms that have accumulated in it, but such partially sterilised soil may subsequently possess a very large bacterial flora, and show a rapid production of ammonia from the more complex organic matter in the soil. Organisms present in the soil as spores, and therefore resistant to such temperatures as 70° C., may increase enormously in numbers in soil in which their natural enemies, minute protozoa, etc., have temporarily been removed by the sterilisation process. This brief reference to a very complicated problem must serve to indicate how complex are the processes at work in a soil, where innumerable organisms of the most diverse types are growing, and in their struggle for survival may indirectly affect profoundly the root systems of the green plants, with the welfare of which we are primarily concerned.

By a system of crop rotations, including leguminous crops, the necessity to manure the soil may be postponed for some time, but eventually it may become advisable to replenish the supply of nitrogenous compounds by the addition of some kind of manure. In districts where stock farming is practised this may be added in the form of dung, which has the additional advantage that the straw and fibrous material usually has a beneficial effect on the texture of the soil, as it opens out and improves aeration in a dense clay soil and knits a sandy soil together. The nitrogenous materials are thus added in the form of complex organic compounds, which are first converted into ammonium compounds by the various micro-organisms and moulds in the soil which accelerate processes of decay. The ammonium compounds may in their turn be oxidised to nitrites by the micro-organism *Nitrosomonas*, and the nitrites to nitrates by *Nitrobacter*. In this way ammonium compounds or nitrates become available for plant growth, and nitrogen in either of these two forms can be utilised. A definite advantage of adding nitrogen in this form is that the soluble salts, in particular the nitrates, are formed only slowly, and consequently a higher proportion is used by the crop before it is washed out in the drainage water. Ammonium salts are held to some extent by the surface film over the soil particles, but nitrates are washed out very quickly, since the nitrogen in the ammonium salts is contained in the positively charged basic part of the salt, whilst in nitrates it is contained in the negatively charged acidic part of the salt. The soil particles are usually negatively charged, and consequently these have an attraction for, and tend to hold, the ammonium ions rather than the nitrate. This effect may be illustrated by the extent to which basic and acid dyes are held, when filtered through a funnel filled with soil. If a solution of the basic dye methylene blue, which is the chloride of a complex base containing the coloured part of the salt, is filtered through soil, the colour base is retained and the filtrate is colourless. On the other hand, if the acid dye eosin, which is the potassium salt of a complex colour-bearing acid group is filtered in the same way, the solution which comes through is coloured and the potassium is retained.

In the absence of cheap supplies of organic manure, or where the effect of the accompanying organic material is prejudicial, it is possible to add the necessary chemical

substances, either as ammonium salts or nitrates. These are examples of the so-called "artificial," although such inorganic manures may be obtained from natural deposits such as the beds of sodium nitrate or saltpetre in Chili.

However, nitrates are also utilised in other processes, such as the manufacture of certain explosives, and one result of the recent world war, when nitrates soared in price and in any case were not available to some of the combatants in the form of natural deposits, was to stimulate investigation of methods of manufacturing nitrates and ammonium salts. Nitrates are formed by the combination of nitrogen and oxygen during every thunderstorm, and this fact has been applied in countries, where water power is cheap, to manufacture nitrates by leading air through an electric discharge. But during the war a catalytic method of forming ammonium compounds was developed by Haber in Germany, and this method has been extended so rapidly that at the present time the main artificial supply of nitrogen is added to the soil in the form of ammonium sulphate. This method involves first the production of relatively pure nitrogen and hydrogen. These gases are then mixed in the proportions of one part of nitrogen to three of hydrogen and are passed over a catalyst, usually platinised asbestos, at a temperature of 800° C., when they combine to form ammonia. The gaseous ammonia is mixed with carbon dioxide and then is passed into a suspension of calcium sulphate, calcium carbonate is formed as a precipitate and ammonium sulphate remains in solution, when it can be decanted off and crystallised. The salt prepared in this way is free from all acid and is particularly suitable for use as a manure.

Nitrogen is the substance which it is most commonly necessary to replenish by manuring in this country, but at times it is also beneficial to add phosphates, which are usually derived from bones, or from the basic slags which are by-products of metallurgical processes. Basic slag has so far proved the most useful manure to the forester; curiously enough nitrogenous manures have proved of little value with forest trees, either in the nursery or in the plantation, but basic slag has often facilitated early establishment of trees planted out on difficult ground. One problem with phosphate manures is their relative insolubility, as a result of which they remain a long time in the soil, but which makes it difficult to estimate their availability

to the plant. Potassium salts are also added, sometimes as the natural mineral deposit kainit.

Every country probably has its own problems of manurial practice ; the laterite soils of the tropics with their different drainage conditions, different population of micro-organisms, fluctuating with the seasons in a different way, will certainly require different treatment from the moist, relatively cold, English soils. South Africa has already found astonishing importance to attach to phosphate manuring, if the sparse vegetation of their relatively dry tablelands is to carry stock. In other countries with special crops, problems of deficiencies or excesses, even of such elements as boron or manganese, have arisen.

Chlorotic vegetation in some regions seems to be due to deficiencies of soluble iron, in other cases it may be remedied by potassium manuring or, in South Africa, even by copper salts. Needless to say, with the complex metabolism of the plant, which again derives its sustenance from the complex system of the soil, the whole question of the application of the scientific study of the entry of inorganic solutes from the soil to the plant, is in a very preliminary stage. In any specific case, local experience, supported by local experiments, is necessary before it is safe to apply general principles to farming practice on an economic scale.

CHAPTER XXXI.

SPECIAL NUTRITION IN FLOWERING PLANTS.

The Saprophytic Habit.

In Chapter XXVII a description was given of the mycorrhizal habit by which some of our forest trees seem to benefit from the close association of their root systems with the filaments of various fungi. This association is thought to be symbiotic, the fungi undoubtedly absorb materials released from decaying organic matter in the ground and some of this material, and also water from the soil, may be passed to the root system. The fungus threads reach to the surface of the soil at certain times of the year and form their reproductive bodies, from which they may be identified as some of the common toadstools of our woods.

This mycorrhizal habit is also very strongly developed in many moorland plants and in orchids. The seedlings of these plants do not develop freely unless the root system is invaded at an early stage by the filaments of an appropriate fungus. It is very suggestive, however, that whereas growers used to rear orchid seedlings with great difficulty, by growing them in soil carefully infected with a suitable fungus, of recent years very successful germination has been obtained on gelatine or agar containing supplies of sugar as well as the necessary inorganic salts. When soluble organic matter is thus supplied to the seedlings they grow well, even if no fungi are present. It is natural to think, therefore, if difficult to prove, that when the fungi are present they must help in the supply of organic matter to the root system associated with them, and this seems to be confirmed by the fact that some orchids, and some dicotyledons allied to the moorland heath plants (*Ericaceæ*), have this mycorrhizal habit very strongly developed and no longer develop a green leaf system. As such plants cannot manufacture their own organic carbon compounds from the carbon

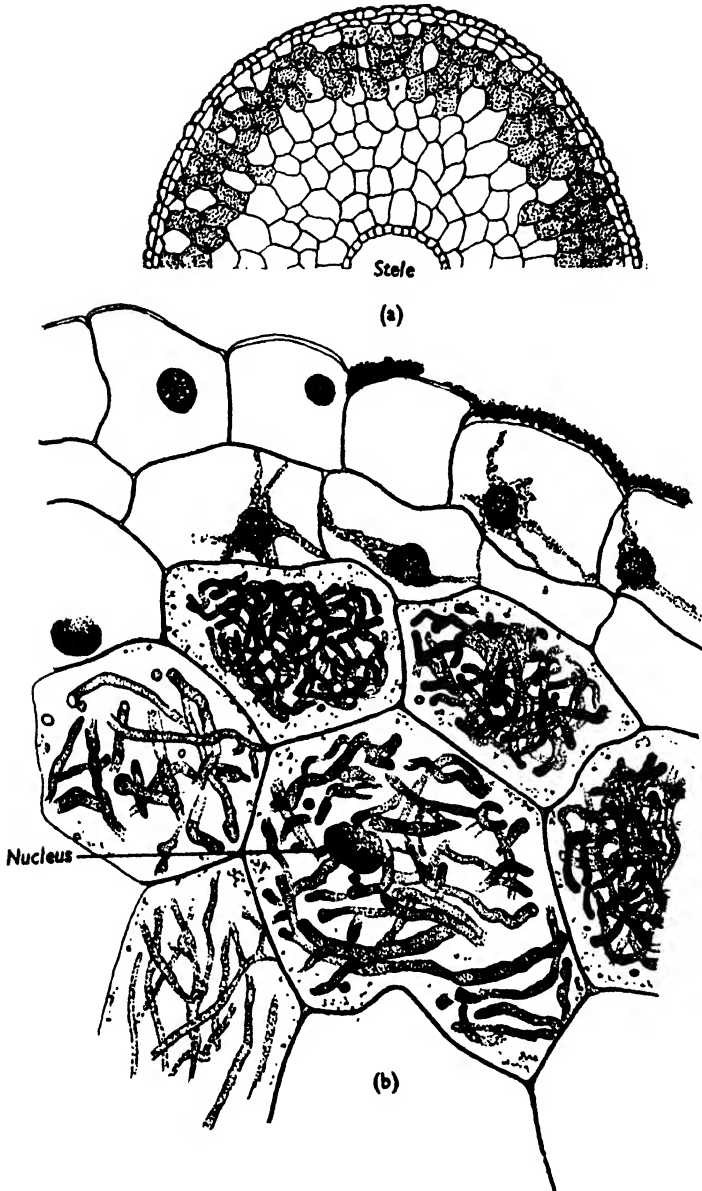


FIG. 113.—*Neottia*, part of a transverse section of the root, (a) showing the position in the cortex of cells infested with mycelium ($\times 30$), (b) a few of these cells ($\times 480$).

dioxide of the air, they must be regarded either as saprophytes, which are able to live on decaying organic matter in the soil through their symbiotic partnership with the fungus, or as parasites which draw upon the food supplies gained from the soil by the saprophytic fungus.

Amongst orchids this habit is well shown by the Bird's Nest orchid, *Neottia Nidus-avis* Rich., and by the coral root, *Corallorhiza trifida* Chatel. The bird's nest orchid bears this name because the thick roots form a dense rounded clump; in these roots

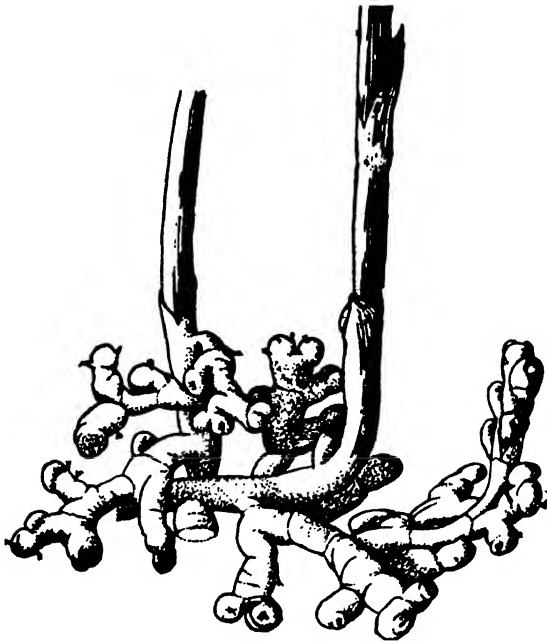


FIG. 114.—*Corallorhiza innata*, rhizome ($\times 1$).

the outer cortical tissues are infested with the hyphæ of a fungus, which freely enter the living cells (Fig. 113), and in many cases are to be seen within them in a state of disintegration. From the root surface the hyphæ ramify into the soil and do not form a dense web over the surface of the root as in the mycorrhiza of forest trees. In *Corallorhiza* no true roots are formed, but the much-branched rhizome has its outer tissues infested with fungal hyphæ in a similar manner (Fig. 114).

Another saprophyte, which is related to the heaths, is the bird's nest, *Monotropa Hypopitys* Cr., a plant found in this

country in the shade of beech or fir woods. The plant is devoid of chlorophyll and bears only pallid scales and flowers on its aerial stems. Apart from these few special cases where the plant depends upon a precarious symbiotic union with a fungus, the saprophytic habit seems undeveloped amongst flowering plants, whilst in the great group of fungi, literally thousands of species are known which derive all their organic nutriment from the dead remains of plants or animals (Chapter XXXII).

The Parasitic Habit.

In addition to the saprophytic species there are countless species of fungi which draw their nutriment from other living organisms, but this parasitic habit is comparatively rare amongst flowering plants. The degree to which it is developed varies in different types, and a comparison of some of these gives an indication of the lines along which this very specialised habit has developed.

In the foxglove family or Scrophulariaceæ, the sub-family of the *Rhinantheæ* includes a number of plants which are normally partially parasitic upon the grasses and other plants amongst which they grow. As a specific example of this group may be taken the yellow rattle, *Rhinanthus minor* L., a plant frequently found in poor grassland. This plant has green leaves and is thus able to manufacture organic food for itself, but through its root system it is also able to supplement its food supplies by parasitising the roots of its neighbours. Yellow rattle is an annual plant, and the seedling soon forms a characteristic branched root system in which, wherever branch root initials begin to grow out, a swollen outgrowth of the cortex of the parent root is formed, with a surface rough with short hair-like outgrowths. In some cases this simply remains as a collar around the base of the branch as it grows out, but if the protuberance comes in contact with the root of some other plant, then this tissue closely encircles the host root, whilst the apex of the branch penetrates the host as a sucker or haustorium (Fig. 115). In the sucker, tracheids are formed which link up with the xylem of the host root, and since the connections to neighbouring roots formed in this way are very numerous, they must provide an efficient means of supplementing the normal food supply. In addition to *Rhinanthus*,

other common plants in the same group with this habit are found in the genera *Euphrasia*, *Pedicularis*, *Bartsia*, and *Melampyrum*. Whilst species of *Odontites*, *Rhinanthus*, and *Euphrasia* are known to be able to survive without a host plant, *Melampyrum* does not make full development without supplementing its food supplies in this way, but since the plants all normally grow in grassland, contacts with neighbouring roots occur freely and the roots are covered with the haustorial outgrowths.

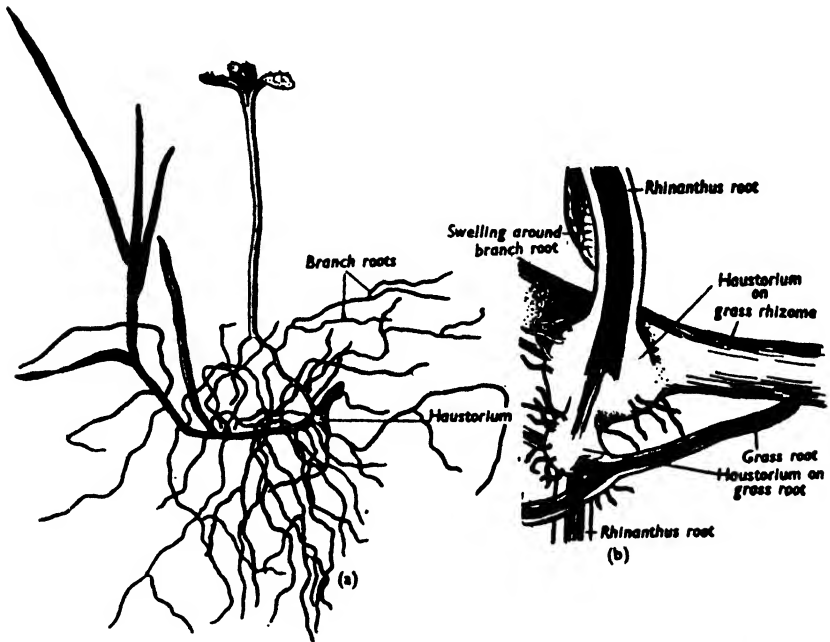


FIG. 115.—*Rhinanthus*, (a) seedling with haustoria on its roots (white) penetrating grass roots (black) ($\times \frac{1}{2}$), (b) enlarged drawing of the haustoria, which are attacking both rhizome and root of the grass ($\times 15$).

An allied genus *Tozzia*, found in mountain pastures on the Continent, and not unlike a large eyebright (*Euphrasia*) in appearance, has pale green leaves, which are obviously deficient in chlorophyll, and indeed the seedling of this plant spends the first two or three years entirely underground; during this time it bears only scale leaves and derives all its food supplies by parasitising the roots of other plants. In this plant we see a transition towards the completely parasitic habit of the allied English plant, the toothwort (*Lathraea Squamaria* L.), which

owes its name to the fleshy tooth-like scale leaves which occur in decussate rows on its underground rhizome (Fig. 116a). In this plant even the aerial flowering shoot bears only whitish scale leaves below the spike of flowers, so that the plant is devoid of chlorophyll and derives all its elaborated food materials from the roots of the host plant. Toothwort is able to parasitise more than one kind of plant, but most frequently in this country it is found associated with hazel. Branched roots arise from the rhizome, and where these are in contact with the roots of the host, haustoria are formed, which consist of a swollen cortex with a central strand of vascular elements in connection with the xylem and phloem of the host root. The swollen scales on the rhizome have a curious structure, for the lower or dorsal surface becomes so completely over-arched that it is present in the adult leaf simply as an internal and richly branched chamber (Fig. 116b). This cavity is lined with glandular structures of two different types; one consists of a one or two celled stalk surmounted by two to four swollen cells with dense contents, in the other type a large lens-shaped cell, which is partly buried in the epidermis, is surmounted by four cells with denser contents (Fig. 116c); between the inner pair of cells in the latter type a small pore is present with the cuticle interrupted above it, so that in surface view these glands are reminiscent of stomata. Below the epidermis is a very loose mesophyll tissue, in which the intercellular spaces are frequently charged with liquid. Sap is released into the leaf cavity, probably having been secreted mainly from the sunken type of gland, and as a result the soil around the rhizomes is usually found to be very wet through the exudation of this liquid from the leaf cavities. The function of these curious leaves is still a matter of speculation, but one thing about which there can be no doubt is that they are storage organs. The large parenchyma cells are packed with strikingly large starch grains, and it is remarkable to find such large quantities of carbohydrate in the leaves of a plant which has had to derive all its supplies of these substances from the host plant upon which it is parasitic.

Closely allied to the toothwort are the broomrapes (*Orobanche* spp.), a family of root parasites with several representatives in Great Britain, most of which have specific host plants. The seeds of these plants are minute, and seem only

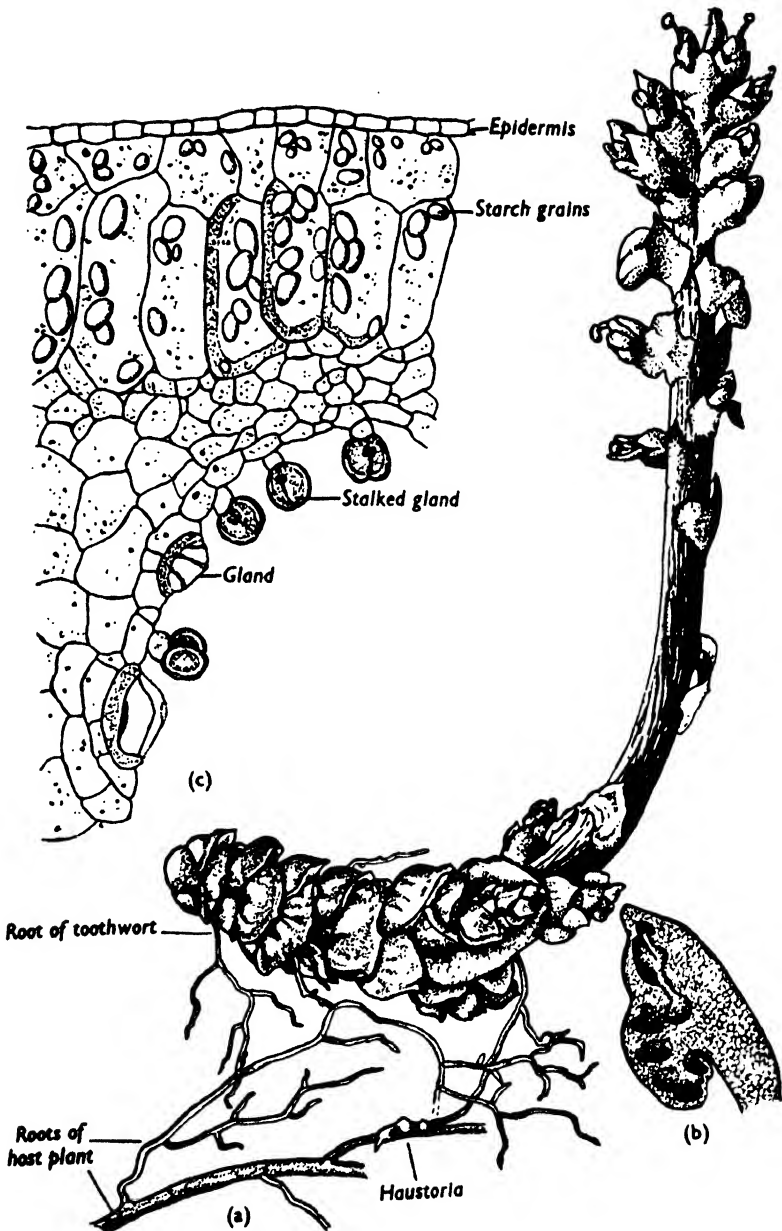


FIG. 116.—*Lathraea*, (a) habit ($\times 1$), (b) section of the leaf showing the branched chamber opening to the abaxial surface ($\times 4$), (c) part of the surface of the chamber showing the two types of glandular structure ($\times 100$).

to germinate if in contact with the roots of a suitable host plant. Of the thread-like embryo in the seed, one end, presumably the root, emerges and immediately enters the tissues of the host plant and establishes an intimate connection with its vascular strand. The remaining part of the embryo grows into a small tuber from which secondary out-growths may arise and also make connection with roots of the host. After a time there arises from the tuber an erect aerial stem which bears only brown scale leaves and brown flowers. After flowering the plant sheds its seeds and usually dies away.

The mistletoe (*Viscum album* L.) illustrates the development of the parasitic habit by a plant of quite a different group of flowering plants, the family Loranthaceæ, which is represented largely by shrubby plants and in which several species show a semi-parasitic habit. The mistletoe produces tough, green leaves on a woody, forked stem, and seems to rely upon its host plant for the water and salts which normal plants derive from the soil through their roots. The plants are dispersed by birds, and the seeds, surrounded by a coating of viscous slime, are left on the bark of trees, to which they become firmly attached as the slime dries. On germination, a curious disc-like structure is formed first, and from this there grows out a fine peg which penetrates by way of a crack in the bark and grows through the tissues of the host until it finally reaches the surface of the wood. From the disc is produced also the leafy shoot, but this at first makes very little growth. The peg formed by the sucker develops a core of lignified wood, and the following year, as the host stem undergoes radial growth, this peg becomes buried in the host wood, but each year from the neck of the original sucker other branches are formed which grow along the surface of the wood for some distance and in their turn become buried by radial growth of the host. The neck of the sucker itself does not become buried, as it has a growing region which is active at about the same level as the cambium of the host, and from this original sucker the mistletoe is continually making new unions with the wood of the host. There is thus built up a sort of "woody crown" in the position of the original sucker, from which there arises the woody shoot externally, and internally the ramifying system of woody strands which run along beneath the bark and then have their tips firmly anchored in the wood of the host, in which they

become buried through its continued radial growth. From the ramifying sucker system other shoot systems may also arise, and so from one original mistletoe infection, several tufts of mistletoe may be developed on the branches.

Quite a different parasitic habit is shown by the dodder (*Cuscuta*), a climbing plant belonging to the Convolvulacæ. Several species occur in Great Britain and parasitise leguminous plants, heather, etc. The seeds are small and relatively late

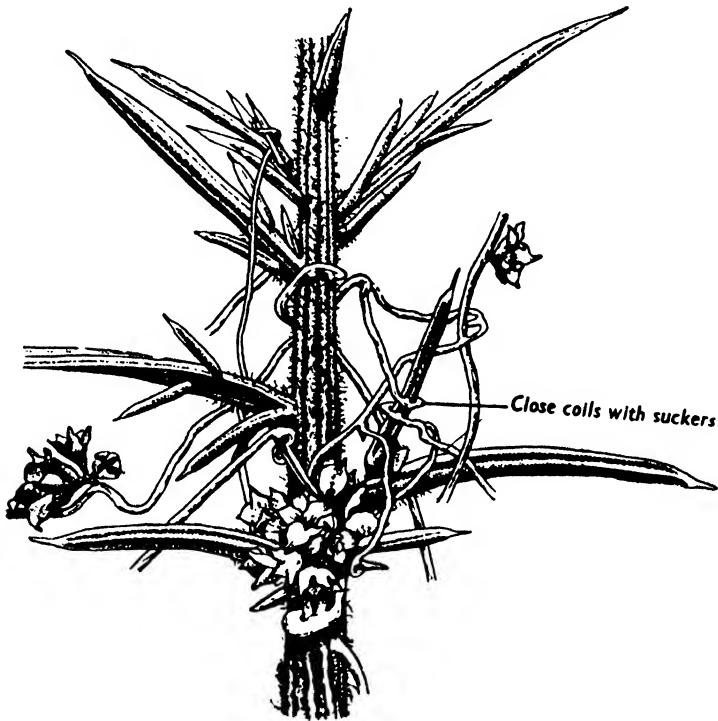


FIG. 117.—*Cuscuta*, attacking gorse ($\times 2$).

to germinate ; each gives rise to a fine white thread, which raises itself into the air and is likely to make contact with the shoot of some earlier growing plant. It immediately twines closely around this for a few turns and on the inner side of the coils there are formed adhesive discs from which suckers grow into the tissues of the host (Fig. 117) Within the sucker a central core of lignified xylem connects with the wood of the host ; around this core, elongated cells ramify in the

phloem so that an intimate connection is initiated between the parasite and both types of conducting tissue of the host. After this first formation of suckers, or haustoria, the dodder plant continues its elongation growth, throwing itself forward at times in long loose coils and then making a few more close coils, bearing haustoria, around a part of the same or a different host plant. Occasionally clusters of small, pale pink flowers are borne along the slender, branching stems and these are surrounded by small bract-like leaves, but the whole plant has no chlorophyll and no anchorage to the soil, and is thus entirely dependent upon its host for its water, salts, and organic food supplies.

Insectivorous Plants.

In addition to the parasitic plants which derive their organic supplies from living plants, there are many cases where members of the animal kingdom may be parasitised. In the case of parasitic fungi all the nutriment of the parasite may be drawn from such a source, but in the more highly organised flowering plants, the only examples of such specialised nutrition are plants which supplement the supplies of organic material made through the agency of their own chlorophyll, by food drawn from the bodies of the insects they capture.

The commonest examples of this habit in Britain are species of *Drosera*, the sundew, which bear a rosette of reddish leaves, the surface of which is almost covered by the mantle of "tentacles," and *Pinguicula*, the butterwort, which has a rosette of leaves of a very pale green colour, indicative of a poor chlorophyll content. Both plants are to be found on moorland peat, where the water supply is more than adequate and the soil as a result very boggy. In this soil the roots are probably ill supplied with salts and especially inorganic nitrogen, so that the additional supplies drawn from the organic material of the captured insects are probably of importance.

In *Drosera rotundifolia* L. the leaves of the rosette have a flattened stalk which expands into a rounded blade, whilst the blade is elongated in *D. longifolia* L. The upper surface of the blade is covered with tentacles, which consist of an

expanded insertion, bearing a shaft which enlarges distally into the capitate head. These are not merely epidermal outgrowths, as each tentacle contains a vascular strand which ends in the head in a cluster of short tracheids. Around the vascular strand in the shaft are long cells with thickened walls and an epidermis with scattered glandular hairs, on the head the epidermal layer consists of densely protoplasmic secreting cells. The water supply is evidently in excess of evaporation, for the heads of the tentacles are nearly always enclosed in a liquid drop which is viscid and sticky, owing to excretion from the surface cells.

The whole leaf then appears covered with shining drops of mucilage, and these are so sticky that when an insect comes in contact with the leaf, it finds it practically impossible to become free again. Such a snared insect probably releases a certain amount of alkaline substance, to which the experiments of Charles Darwin have shown that the turgid cells of the shaft are exceedingly sensitive, particularly to the ammonium ion. Thus if a drop of dilute ammonium carbonate is placed on the head of a tentacle, the secretion immediately becomes much more copious, as it does also when an insect or a piece of raw meat or white of egg come in contact with the glandular head. This greater secretion suggests a change in the permeability of the cells which also seems very probable from the movement which follows in the tentacle. Over the central part of the upper surface of the blade the tentacles are short and straight, whilst around the margins they are much longer and asymmetric in structure, with the glandular surface only developed adaxially. When a marginal tentacle is stimulated, either by direct contact or indirectly by stimulation of other tentacles of the leaf, the release of substances and the resultant changes in turgidity lead to considerable relative changes in length of the cells on the two sides of the shaft. This causes the tentacle to bend over until its head is brought down on to the upper surface of the leaf and, since this happens with most of the marginal tentacles, any insect originally snared in the viscid drops of liquid, either on the marginal or central tentacles, is finally held down on the surface of the leaf and buried under the clustered heads of the marginal tentacles. The secretion includes acid and various enzymes, particularly a protease, and the tentacles remain bent over

during digestion and until the soluble products of the insect have been exhausted. Then the cells on the inner side of the tentacles extend so that they open out once more and the leaf is ready to react to another victim. The movement in response to contact is slow and is initiated as the result of a definite chemical stimulus released into the liquid on the head of the tentacle. No movement takes place on contact with any object which does not release suitable substances into this fluid. As tentacle movement is a growth phenomenon, it cannot be repeated indefinitely and it seems that a tentacle can only respond to stimulation about three times.

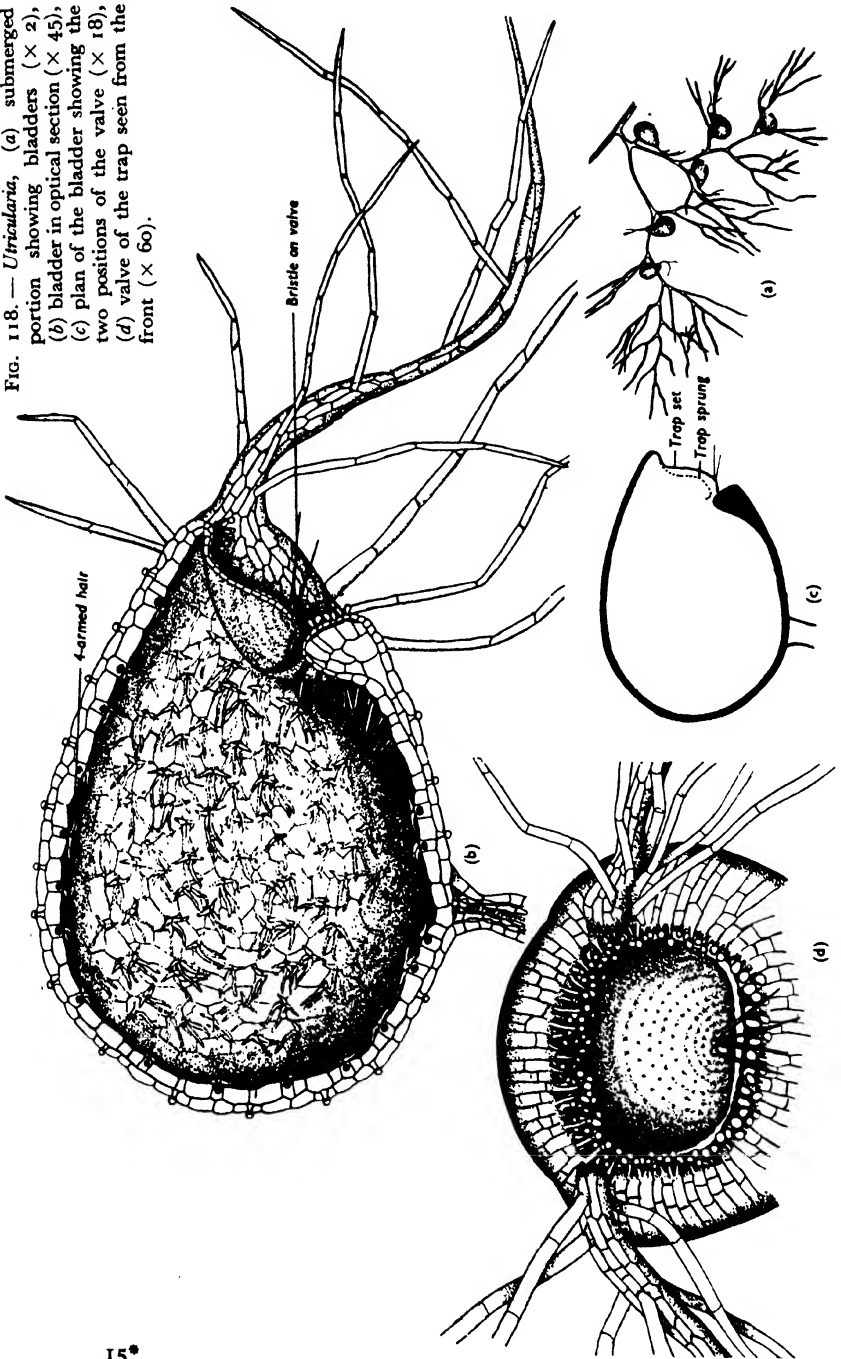
With the mechanism in *Drosera* may be compared that of the Venus' fly-trap (*Dionaea muscipula* Ell.), a native of American bogs which can only be grown under greenhouse conditions in this country. The stalked leaves are borne in a rosette and the blade has a very strong hinge in the mid-rib region; it seems evident that the leaf tissues must be very turgid and their contents in a state of very delicate balance, because when some fine bristles present on the upper surface of the blade are touched (two contacts in quick succession are usually necessary), the upper surfaces snap together, moving on the hinge, whilst rows of fine, comb-like teeth on the margins interlock. The whole movement is so excessively rapid that if the trigger mechanism has been released by contact with an insect, this latter is frequently imprisoned, the glandular hairs on the upper leaf surface then release their secretions and the leaf remains closed until the insect is digested. If the movement has been caused by contact with some neutral foreign body, such as a piece of stick or rock, the leaf soon opens again and the trap is reset.

The butterwort, *Pinguicula*, is represented by several species, all of which are rosette plants growing in bogs. On the upper surface of the leaf are innumerable short, capitate, secreting hairs, and other larger hairs on longer stalks. Small insects are caught and digested on this sticky surface and there also appears to be a tendency for the leaf margins to roll inwards by differential growth when insects have been caught.

Two other types of insect-catching mechanisms found in flowering plants deserve brief mention, though only one of them is exhibited by a British plant, the bladderwort, *Utricularia*. Though belonging to the same family as the butter-

wort, the bladderwort is a plant of very different form ; all the species occur in ditches, pools or very wet bogs, and the finely divided vegetative system, which is difficult to classify either as divided leaf or branching stem, is usually totally submerged and only the inflorescences, with their yellow flowers, rise above the water. Upon the sub-divisions of the green branched system occur little bladders (Fig. 118*a*), in which small insects are often found entrapped. The bladder is oval and attached to the vegetative body by a short, slender stalk. Seen from the side, the bladder is rather more pointed at the end distal from the stalk, and here a mouth-like opening is present, which is closed by a delicate valve-like "door" (Fig. 118*b*). To the sides of the mouth are branched hairs, whilst on the valve itself are a few straight, stiffer bristles (Fig. 118*d*). The valve is attached along the dorsal margin of the mouth and some way down the sides, but is free towards the flat face of the bladder, where it rests against a thickened ledge projecting from the inner surface (Fig. 118*c*). In the normal way the bladder is completely closed by the "door," the margins of which are also sealed by mucilage and an over-lapping flange of loose cuticle. For many years the mechanism by which animals are entrapped in these bladders was not understood, but recently this has been demonstrated quite independently in different countries. It is found that the liquid which initially fills the bladders is pumped out of them, presumably by the action of curious, four-armed hairs which line the bladder cavity in great numbers. These structures are thought to absorb the liquid, which must then either be pumped out through short capitate hairs which are present in the outer half of the wall, or possibly it may pass away along the vascular bundle in the stalk of the bladder ; in any case the liquid is absorbed to such an extent that the elastic sides of the bladder become drawn in so as to make the bladder very narrow when viewed from the front. The valve door is then in a condition of very delicate balance, and if even a very minute insect comes in contact with the bristles on the valve, the latter is apt to buckle in and slip over the ledge, and thus allow a sudden inrush of water to relieve the tension ; this inrush carries with it the insect. The valve then slips back into its original position and the insect is trapped. After the trap has been sprung in this way, the

FIG. 118. — *Utricularia*, (a) submerged portion showing bladders ($\times 2$), (b) bladder in optical section ($\times 45$), (c) plan of the bladder showing the two positions of the valve ($\times 18$), (d) valve of the trap seen from the front ($\times 60$).



process of absorption becomes active again and, within about half an hour or less, the trap is again ready to catch further prey. The animals seem to live for some time in the bladders but eventually die, and as the remains decay they are presumably absorbed and used in the nutrition of the bladderwort.

Remarkable pitcher mechanisms for trapping insects are found in two widely separated parts of the world ; species of *Nepenthes* occur in the Malay Archipelago as shrubby epiphytes or climbers, so that they may be living under conditions where normal nitrogen supplies are deficient ; species of *Sarracenia* are found in the peat bogs or muskegs of Northern Canada, and parts of tropical and sub-tropical America, and growing in conditions where the supplies of inorganic salts of nitrogen are certainly deficient.

In *Nepenthes* the leaves are very long, with a basal, blade-like expansion which continues as a tendril that twines around some support before it expands again to form the pitcher. The pitcher is a remarkable urn-shaped structure, often several inches in length, and may be roofed over by a stalked lid formed by an expansion of the tip of the leaf. The lower portion of the inner surface of the pitcher is covered by numerous multicellular secreting hairs, and the pitcher is often half-full of secreted liquid. In this liquid there may be found astonishing quantities of decaying insects, which have evidently found their way into a trap and have been unable to escape.

Glandular hairs are also present about the mouth of the pitcher, and it seems possible that insects are attracted to the pitchers either by the secretions of these or by the bright colour of the pitchers. The visiting insects alight on the rigid rim of the urn, but the surface here is extremely smooth and apparently even wingless ants, which can walk on vertical glass surfaces, are unable to keep their footing on it. This appears to be due to a waxy coating over the smooth epidermal cells, built up of minute scales which readily detach themselves from the wall when the adhesive papillæ of the insects press upon them. The adhesive cushions which usually enable insects to walk on smooth surfaces are thus thrown out of action, and, on the smooth surface, claw-like feet are equally useless and will not even hold the insect whilst it cleans the waxy scales off its adhesive cushions. The insect then falls into

the liquid below or on to the glandular secreting surface, and in either case is prevented from escaping by the presence of a forest of downwardly directed, epidermal hairs, just beneath the rim of the pitcher. On death the insects are hastened in their decay by digestive enzymes secreted into the liquid, and the soluble products are then probably absorbed by the wall of the pitcher.

In *Sarracenia*, and other members of the same family, the whole leaf is modified to form a long tubular pitcher, the opening of which may be partly arched over by the distal part of the leaf. Short, epidermal cells, near the upper margin of the pitcher, secrete a sugary liquid which appears to be an attraction to insects, and again the very smooth inner surface of the upper part of the pitcher causes many of the insects to fall into the interior. The smooth, epidermal surface in this case is very differently constructed from that of *Nepenthes*, and consists of cells which overlap one another like the tiles of a roof, but the result seems to be the same in either case and ants or other insects are unable to stand on this inner surface. In this case the inner surface of the lower part of the pitcher does not show much evidence of the secretion of liquid by secreting hairs, but there seems little doubt that the substances released by the decay of the insects are absorbed by the pitchers. Indeed without this added source of supply it would seem doubtful whether some of these pitcher-forming plants produce a sufficiently wide assimilating leaf surface, though down the side of the pitcher, towards the centre of the rosette of leaves, a wing-like expansion usually increases the assimilating surface.

CHAPTER XXXII.

FUNGI AS PARASITES AND SAPROPHYTES. STERILISATION AND THE TECHNIQUE OF PURE CULTURE.

FEW of the highly organised flowering plants obtain their food supplies by other means than photosynthesis, but amongst the more simply organised plants, the very large group of fungi contain no chlorophyll and must live either as saprophytes or parasites. In the nineteenth century methods were developed which made it possible to grow these organisms separately on suitable nutrient media, and since then their study has become the specialised task of the mycologist, plant pathologist, and bacteriologist. In these pages we can only pass in review a few selected types, which will serve to illustrate the practical importance that attaches to their study, and to indicate some of the interesting problems that arise when their life histories are studied. A few simple exercises with these plants will also show something of the general principles which form the basis of methods for their isolation and cultivation.

A Rust Fungus.

Many of the epidemic diseases which spread havoc amongst cultivated plants are due to parasitic fungi, or unicellular parasitic organisms such as the bacteria, or to still more obscure disease producers or pathogens, known as viruses, which are thought to be minute organisms of so small a size that they defy the resolving powers of the microscope, and pass through all but the finest filters.

It will be best to take for study one of the more highly organised types of fungus parasite, such as a rust fungus, so-called because they produce, on the surface of the plant attacked, masses of fine spores, which collectively give the appearance

of streaks, blotches or specks of a rusty colour. The life cycles of these fungi are comparatively complex, owing to the variety of spore forms produced, but they show exceptionally clearly how a specialised, parasitic organism may be most perfectly fitted for this mode of life. Indeed the rust fungi are so specialised as parasites that they are never found growing except upon a living host, and so far it has proved practically impossible to cultivate them for any length of time away from it; such parasites are described as obligate, as compared with facultative parasites, which sometimes live on dead organic matter, sometimes on living hosts.

Besides being obligate parasites, the rust fungi are usually very restricted in the host plants they are able to attack; some are able to pass the same stage of their life cycle on several genera within a family, others are localised to the species of one genus, whilst there are also forms of rust which appear to be restricted to particular varieties within a species.

The rust which has received most attention, on account of its importance as an economic disease, is the stem rust of cereals (*Puccinia graminis* Pers.). This was certainly known in Roman times, when a special day was set apart for prayer and sacrifice that the crops might be spared from rust, and very probably it was known very much earlier, though the nature of the blight was not of course realised until centuries later. By the eighteenth century it was noticed that the attacks of rust on the cereals were associated with the presence of barberry bushes in the vicinity, and on 13th June, 1760, a law was passed in Massachusetts ordering the destruction of all barberry bushes. The full explanation of this fact was not discovered until 1865, when de Bary showed that the minute spores, or unicellular reproductive bodies, of the rust on cereals, could produce the disease on barberry leaves, and the spores from the barberry leaves could re-infect the cereal. This possession of two host plants, on each of which a species of rust spends part of its life cycle, is now fully confirmed and applies to many species, though some appear to be able to continue to survive without completing the full life cycle involving two hosts, whilst others are able to pass through their complete cycle on the same host.

Owing to the greater amount of research which has been carried out upon the stem rust of cereals, *Puccinia graminis*, the account which follows applies strictly to that species, but

in many cases the points recorded have been found to apply to other species too, and are probably of general application.

The infection of barberry (*Berberis vulgaris* L. or *Mahonia Aquifolium* Nutt.) occurs in the spring through the agency of minute, thin-walled, air-borne spores. These are so small that they only provide for very limited growth by the germ tube, but this immediately pierces the cuticle of the host and gains access to further food supplies. The penetration of the cuticle seems to be mechanical, and this method of entry restricts the attack of the invading fungus to the surface of young leaves, where the cuticle is thin. Once inside the host tissues, the fungus forms a web of fine, septate threads, or hyphæ, with uninucleate cells. These hyphæ ramify between the cells of the host, at places sending short branches or haustoria into the cells, by means of these the parasite withdraws its nutriment (Fig. 119*b*). The hyphæ, collectively known as the mycelium, tend to spread in all directions from the point of infection. Sections mounted in concentrated chloral hydrate solution, coloured with a few drops of acid fuchsin solution (2 per cent. acid fuchsin in 70 per cent. alcohol), show very well the mycelium in the tissues. The infection often causes some malformation of the leaf, and in some species the fungus may affect the chlorophyll content of the host and also cause a local accumulation of starch.

In certain places the mycelium forms aggregations beneath the epidermis, most frequently the upper, and from this mycelial bed arise numerous thin, erect branches which press against the epidermis, rupture it and protrude as a small tuft through the hole. The whole structure, the spermatogonium, produced in this way has, in cross-section of the host leaf, the outline of a flask-shaped cavity, lined by sterile hyphæ, but enclosing shorter hyphæ which bud off distally minute round or oval spores, known as spermatia (Fig. 119*a*). The spermatia are exuded through the mouth of the spermatogonium in a drop of sweet-tasting nectar, which, in some species, also has a characteristic odour.

The same mycelium which has formed first the spermatogonia in the central region of the young infection, also extends through the leaf and forms, mainly around the periphery, localised masses of mycelium just to the inside of the lower epidermis and from these arise other cup-shaped structures,

the æcidia, which occur in groups, known as cluster cups. The cells which give rise to the spores in these cups have

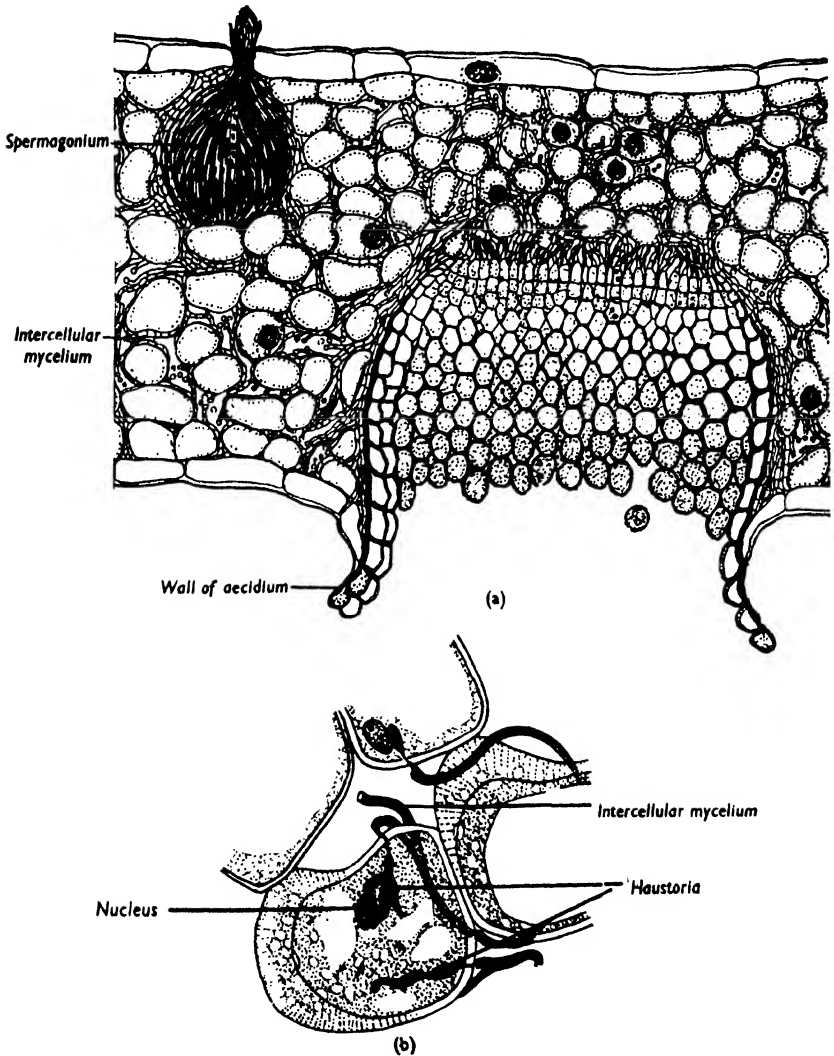


FIG. 119.—(a) Leaf of *Allium ursinum* in section, showing a spermatogonium and an aecidium of *Puccinia Winteriana* ($\times 65$), (b) a few cells of the host showing haustoria penetrating the cells ($\times 900$).

become binucleate, and, associated with this change, the whole structure is much more massive than the spermatogonium and the binucleate spores are larger than the spermatia (Fig. 119a).

The binucleate cells near the floor of the æcidial cup bud off a succession of cells, each of which also cuts off a small sterile cell to the inside whilst the outer cell becomes the æcidiospore ; in this way numerous long chains of spores are formed, so closely packed against each other in the æcidium as to give them a polygonal outline, and finally the pressure causes the rupture of the epidermis and the exposure of the æcidiospores in a shallow and widely open cup ; this is seen clearly if the leaf is examined under the microscope by reflected light. The distal cells of the chain are the oldest ; they round off and readily separate owing to the collapse of the small sterile cells between the spores. The rounding off and release of the æcidiospores may take place with considerable force, so that the spores are shot off to a distance of 1-15 millimetres from the leaf. These spores have orange contents, the wall is slightly warted and in the inner wall layers a few thin places, or germ pores, are present.

The sudden swelling and release of the æcidiospores is likely to take place under moist conditions, and if under these conditions they fall upon a leaf, the spore soon germinates. These larger spores can make more growth on the material in the spore and grow over the surface of the leaf until a stoma is reached, when the tip of the hypha immediately turns in through the pore and then swells, thus securely anchoring the fungus to the leaf. If the leaf is not of a suitable host, the fungus makes little progress, but if it is a cereal, the mycelium ramifies between the cells, sending haustoria into them in places. This mycelium, like the æcidiospore from which it arose, is built of binucleate cells, in places it develops localised aggregations of mycelium which rupture the host epidermis and produce crops of spores (Fig. 120a). Each fertile hypha forms an elongated stalk cell, from the end of which a rounded uredospore is formed ; each uredospore is a binucleate spore with rust-red contents, enclosed in a finely warted wall with a few germ pores. The uredospores readily separate from their stalk cells and if they fall upon a leaf, where the rough wall may help to maintain contact until germination, they germinate like the æcidiospores and simply produce a new infection of the fungus, bearing either further crops of uredospores or the next spore stage. It is the uredospore stage which is mainly responsible for the

rapid spread of the disease ; it is also the rusty colour of this stage which renders the disease conspicuous, so that very commonly the host plant upon which this stage of the fungus is found, provides the specific name of the rust species.

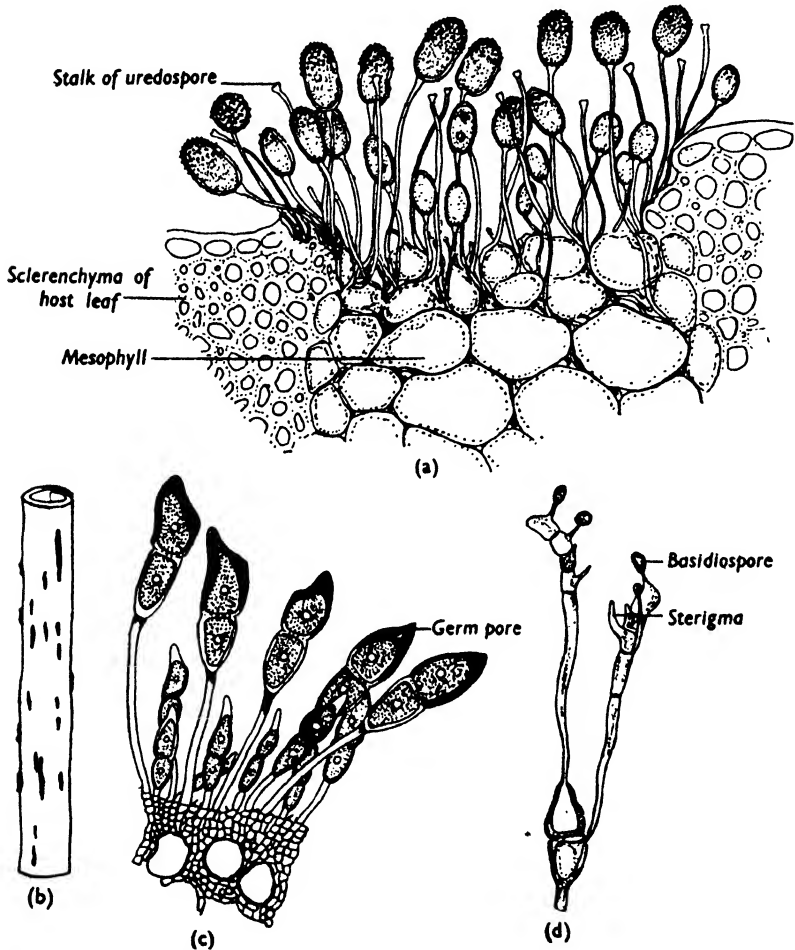


FIG. 120.—*Puccinia graminis*, (a) section through a pustule of uredospores on wheat ($\times 170$), (b) pustules of teleutospores on a leaf sheath of wheat ($\times 1$), (c) section through a pustule of teleutospores on wheat ($\times 230$), (d) germinating teleutospore (after Tulasne) ($\times 150$).

After a time the patches of mycelium which have been producing uredospores, begin to form the next type of spore, the teleutospores, a change which is evidenced by a change

of colour from rust-red to dark brown or black. The teleutospores are also borne on stalks, each forms a two-celled structure or double spore, the lower cell tapering to the stalk, the upper to a pointed apex (Fig. 120*b* and *c*). The young teleutospores are binucleate like the mycelium, but as they mature the paired nuclei fuse so that each ripe teleutospore cell is uninucleate. The wall becomes very thick and dark brown in colour, but about two thin germ pore regions are present in each cell; in the condition of these thick-walled teleutospores, the rust survives the winter, the spores on the ground or on the straw forming a source of infection for the next spring.

On germination the teleutospore sends out from each cell a delicate germ tube; the nucleus formed by fusion in the teleutospore undergoes two rapid divisions in succession, a type of division known as reduction division and to which more detailed reference will be made in Chapter XXXVII. Walls are then formed to divide the structure into four uninucleate cells, from each of which a small pointed outgrowth (the sterigma) is developed, which buds off distally a small, thin-walled basidiospore (Fig. 120*d*). This whole structure, produced on germination of the teleutospore, may be compared with the spore-bearing cells, which also bud off four basidiospores, on the gills or lamellæ seen on the under side of the fruiting bodies of fungi, such as the mushrooms and many of the toadstools. This similarity of the rust fungi and the larger gill-bearing fungi has led to their grouping together in the same section of the fungi, the Basidiomycetes.

It was with the infection of young leaves of barberry by these minute basidiospores that we commenced to follow the life cycle of *Puccinia graminis*. Two points which were left at the earlier stage of the description were the rôle of the spermatia and the way in which the mycelium changes from the uninucleate condition beneath the spermagonia to the binucleate beneath the æcidia. It has been shown that if an infection of barberry is carried out with a single basidiospore, the resulting mycelium produces spermagonia, but does not proceed to the development of æcidia, but where the infection has been from two spores, so that the mycelium from one spore will overlap that produced from another, in about half the cases æcidia will be developed. It is evident that the basidiospores are of two kinds which, since

they cannot be distinguished by any visible features, may be designated + and -, and it is when mycelia of + and - strains come in contact and migration of the nucleus takes place from a cell of one strain into that of the other, that the binucleate condition, necessary for the development of æidia, is established.

The spermatia do not appear to be capable of producing a new infection, but experiment has shown that the introduction of spermatia, from other spermagonia, on to the spermatogonium of a sterile, one-spore infection, enables the change from the uninucleate to the binucleate condition to follow. In nature the spermagonia are visited by flies and other small insects which feed upon the nectar exuded with the spermatia, and are probably also attracted by the characteristic odours in some species, and as they pass from leaf to leaf they certainly bring about the mixing of the spermatia and thus "fertilise" the infections.

Some consideration of this brief survey brings out the remarkable degree of specialisation of the fungus to its host. The essential of such a relationship, in which an obligate parasite is concerned, is that the fungus should be able to get food from its host without killing it, and this is true of the rust so long as the infection is of the right host species; if the wrong host is infected, some cells in the region of infection may be killed, and such dead cells bar the further progress of the obligate parasite, or the host plant may be sufficiently vigorous to form a layer of cork isolating the infection. On the other hand, a facultative parasite has the habit, like any saprophyte, of releasing from its cells enzymes which digest the complex organic substances present in dead cells; if it successfully invades a host, the hyphæ advance through the tissues, killing the cells in their progress, and then live saprophytically upon the contents of the dead tissues. It is still more remarkable to find particular stages of the life cycle of a parasite passed on different host plants, which are usually quite unrelated; this may, however, be very advantageous to an obligate parasite, especially if one of the hosts should be an annual and therefore not always available. Certain epidemic diseases of man, produced by parasitic protozoa, also have alternating hosts; in malaria the organism passes part of its life cycle in the mosquito and part in man, whilst the organism of sleeping

sickness alternates between the tsetse fly and warm-blooded animals, including man.

A further point which emerges in connection with the rust fungus is that the organism, obtaining all its nutriment from its host, may be of extremely simple vegetative organisation. On the other hand, reproduction is extremely prolific, very large numbers of spores being produced, and the complex array of spore forms would also appear to have special significance, being either thin-walled types which germinate at once during the spring and summer when the disease is spreading rapidly to active growing hosts, or thick-walled types formed at the close of the growing season, when the parasite may lie dormant until the return of its host plants to a suitable condition to parasitise.

The stem rust of cereals is not at the present time such a prevalent disease as in earlier times, and sometimes it is difficult to obtain material of this species for examination, especially the stage on barberry. However, the spore types of many of the commonly occurring rusts are so similar in form that they may be examined instead. The æcidial stage of *Puccinia Poarum* Niels. is conspicuous on coltsfoot in May or June, and again in July or August, as this species completes its cycle twice in the year. The uredospore and teleutospore stages are less conspicuous but are usually to be found on the various species of meadow grass, *Poa*, in the neighbourhood of infected coltsfoot. *Puccinia Menthae* Pers. has all its spore forms on wild or garden species of mint and is also a convenient species to examine. There are, however, many others which are likely to be found and which will serve equally well.

Saprophytic Types.

Amongst the vast numbers of species of saprophytic organisms are many very varied types of which the filaments flourish on occasion in the soil. Here, with the bacteria, they play an indispensable rôle; all the time the soil is receiving vast quantities of the remains of organic beings, both dead animal and plant material, and if it were not for the organisms of decay or putrefaction, these materials would collect to such an extent that life would soon become impossible for all other organisms. The activities of these saprophytes resolve the dead tissues

once more into simpler and more diffusible forms, the proteins of plant and animal become broken down finally to ammonium salts, the celluloses and starches of the plant tissues to carbon dioxide and water. The chemical changes thus produced in the substrate by the saprophytic organism are both complex in character and manifold in kind, they are brought about through the release into the substrate of different enzymes from the cells of the fungus, and it is entirely due to the activity of the putrefactive organisms that most soils contain only a proportion of organic humus and not vast masses of intractable, complex organic residues.

The fine hyphal threads, forming the mycelium of the putrefactive fungal organisms, ramify in the material they are decomposing; in this form they all look much alike, but when they reproduce they come above the soil, some bearing toadstool-like, spore-bearing structures, or sporophores, whilst in others the sporophores may be of very varied forms. Altogether there are very numerous species of saprophytic fungi, and many of these have related species which are facultative or obligate parasites. Amongst these numerous saprophytic species, many have such minute spore-bearing structures that in the soil they are usually passed by unheeded, but if food material is left exposed, the spores of such organisms, floating in the air, may be deposited on it; they germinate and form a mycelium spreading through the material, and soon such a prolific crop of spores is produced on the sporophores in the air, that the mould is brought prominently to notice. Examples of such moulds are the green or bluish sheets found on cheese, moist bread, jam or even damp boots, others form a fine cottony growth over the food materials which become covered with black dots as the spore-producing bodies mature. *Mucor Mucedo*, the pin mould, is an example of the latter kind which will usually appear if moist bread is left standing in a warm, dark place, and the following observations would fit a typical occurrence of such a mould. It must be borne in mind, however, that there are many species of these moulds with characteristic differences, and if one wishes to be certain that the species studied is actually *Mucor Mucedo*, it would be advisable to obtain a culture of this species from a laboratory in which moulds are grown in pure culture.

The hyphæ of *Mucor* form a much-branched mycelium,

which ramifies in the substratum ; it is almost non-septate in the vegetative part so that the protoplasm, containing very numerous nuclei, is continuous throughout the plant. Reproductive branches grow erect into the air and swell at the tip, which becomes packed with cytoplasm, nuclei, and food material. This region is then cut off from the stalk by a dome-shaped wall, the columella, so that the end of the stalk protrudes into the cavity of the spore-bearing cell or sporangium. The contents of the sporangium then cleave into a number of parts,

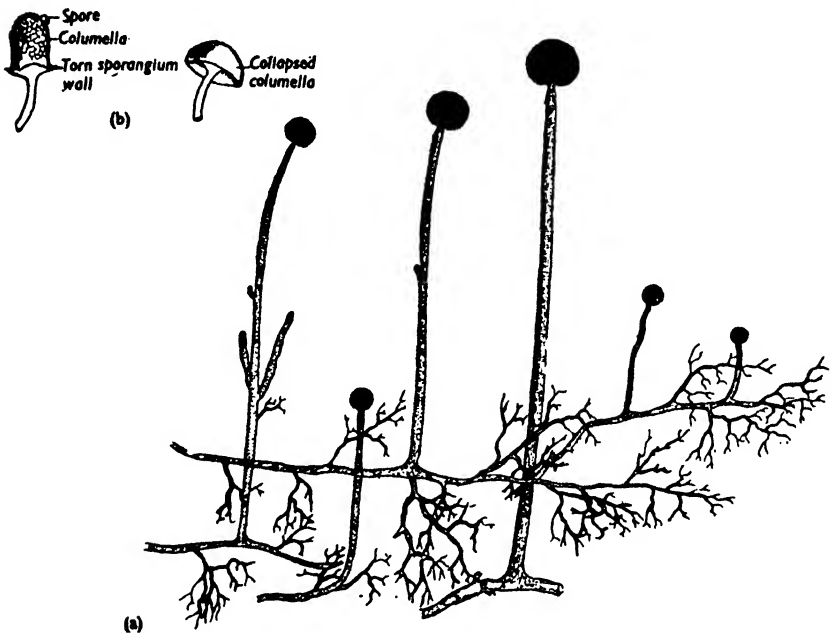


FIG. 121.—*Mucor Mucedo*, (a) mycelium bearing sporangia ($\times 50$), (b) burst sporangia ($\times 70$).

which become separated by wall formation until ultimately a very large number of multinucleate spores are formed ; these round off from one another, and the wall also darkens in colour so that the ripe sporangia look black (Fig. 121a). At this stage the liquid in the stalk increases in amount and the pressure set up in the columella causes the rupture of the thin sporangium wall, which has also tended to dry as all the protoplasm has been withdrawn from it into the spores. The small, colourless spores are readily wafted away by any air currents, whilst the

turgid columella, with a collar of the burst sporangium wall, persists for some time (Fig. 121*b*). This is the common method of rapid propagation of *Mucor* and may be observed in any naturally occurring type. The sporangia show well by reflected light under the microscope; for examination in transmitted light they should be mounted in a mixture of glycerine and 70 per cent. alcohol.

In another method of reproduction occasionally found, two short branch hyphæ, a little stouter than the ordinary vege-

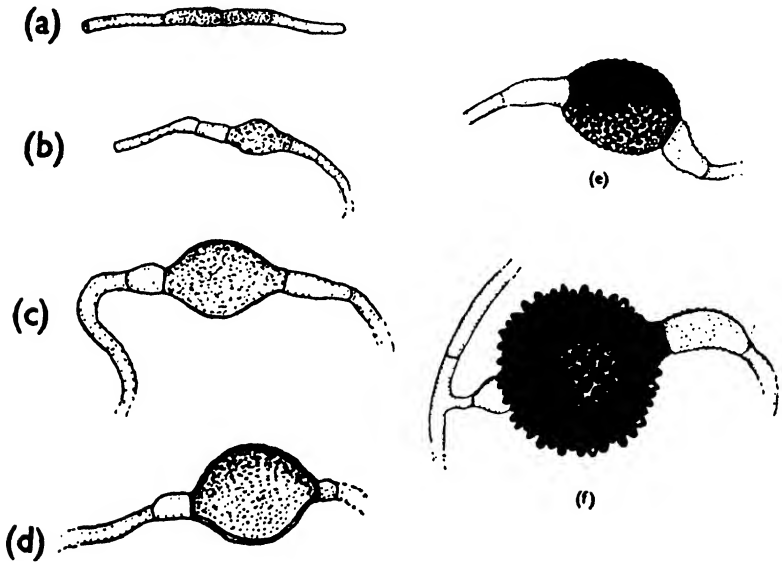


FIG. 122.—*Mucor hiemalis*, stages in conjugation ((a), (b), (c) and (d) $\times 950$), ((e) and (f) $\times 475$).

tative hyphæ, come in contact by their tips (Fig. 122*a*). A cell is then cut off from each by a cross wall, and these cells fuse together by solution of the walls in contact, the cytoplasm becomes confluent and the nuclei introduced from the two cells fuse in pairs. This process seems comparable with some kind of sexual fusion, so that the two fusing cells or gametangia are said to conjugate, and the resulting fusion cell develops into a zygospore (Fig. 122*b-d*). The developing zygospore swells up into a rounded structure, darkens in colour and develops a thick, warty wall (Fig. 122*e-f*); it is a resting structure and usually

does not germinate until several months have elapsed. On germination the thick wall ruptures and the contents grow out enclosed in an inner wall layer to form a stalked sporangium, the spores from which grow into normal vegetative mycelium. During the germination of the zygospore a reduction division of the nuclei takes place to compensate for the fusion of nuclei in the young zygospore.

Very commonly *Mucor* fails to produce zygospores and the explanation of this has been obtained through pure culture work. By the development of these methods, it is possible to obtain a mycelium of the fungus grown from a single spore, and so to compare the behaviour of mycelia from different spores of the same sporangium or from different sporangia. By these methods it has been shown that in most species of *Mucor* there are different strains, though these cannot be distinguished by any visible characters. Zygospore formation never occurs in a mycelium of single spore origin, nor when the mycelia grown from different spores from the same sporangium come in contact, as these are all of the same strain, but if mycelia of different strains are grown side by side in the same glass culture vessel, a black line of zygospores, visible to the naked eye, forms along the line of contact. As no difference can be seen between the two strains or between the two fusing gametangia, they are usually designated as + or - strains, and the formation of gametangia and the subsequent development of zygospores is now known to occur only if mycelia of + and - strains come in contact.

It is most important to a saprophytic organism of this kind, which depends for its food supply upon local occurrences of moist organic remains, that its vegetative growth should be rapid, so that spores may be produced before the food supply is all utilised, and certainly rapid growth and prompt production of enormous numbers of air-borne spores are striking features of such a mould as *Mucor*. Though many of the spores will probably fail to fall upon media suitable for growth, the number produced is so enormous that some will always be present in the air, some will be deposited on any food left exposed and these will soon cause the rapidly spreading process of decay to set in; in fact whenever a medium is available for life, living organisms will soon appear in it.

Before the life histories of these types were known, and before

it was realised that organisms or their spores might be of such microscopic, or even sub-microscopic size, this inability to keep life from generating, apparently spontaneously, in any medium capable of supporting it, led to much discussion. With microscopic technique as yet not far developed, it was not an easy matter to provide convincing evidence that decay only commenced when spores, often sub-microscopic in size, were disseminated into the nutrient medium from the air, and the difficulties that faced Louis Pasteur (1822-95) in establishing this view on a firm experimental basis, against the prejudices and theories of many of his contemporaries, are vividly portrayed in one of the classical biographies of science, "The Life of Pasteur," by his son-in-law, Vallery-Radot.

Starting from the knowledge that fermentation was caused by living organisms of small size, Pasteur had the conviction, the truth of which he was able to establish, that putrefaction was of a somewhat similar nature and also caused by living organisms, and one of his great achievements was to show that, if sufficient precautions are taken to prevent the entry of air-borne spores, a nutrient liquid, once rendered sterile, will remain free from growths of living organisms indefinitely. In order to obtain the sterile liquid it is not sufficient to raise the temperature to a point at which the living and growing organisms are killed (about 70° C.), though this process is still used to increase the keeping qualities of milk and is usually termed pasteurisation. This temperature kills many organisms, but will not destroy some spores in which the living protoplasm is present in a relatively dormant and dry state; it is then more resistant to heat and in some cases even to the temperature of boiling water.

The normal method of sterilising a nutrient medium is to seal the mouth of the flask with a plug of non-absorbent cotton wool and then to heat it in a steam steriliser to 100° C. for about fifteen minutes. It is then allowed to cool for twenty-four hours, when any spores present will germinate and, in an ample supply of nutrient material, will grow vigorously, so that if the liquid is then again heated to 100° C., all these organisms will be in an actively growing and non-resistant condition and so will be killed. This second heating should completely sterilise the liquid, but in order to be quite sure the process of cooling and heating is repeated once more. Liquids thus

heated and protected remain sterile indefinitely—a constant witness against the once popular theory of spontaneous generation. The cotton-wool plug closing the flask must be kept dry, so that before the heating in the steam steriliser it is covered with tinfoil or parchment. So long as the plug is dry, the cotton wool is sufficiently closely packed to filter out any air-borne spores, but spores may germinate on a moist plug, and the living hyphæ of a mould would then grow down amongst the cotton threads and release spores to the inside of the flask.

Pasteur showed that with proper precautions material might be retained sterile indefinitely, and thus laid down the general principles of procedure for sterilisation, upon which Lister subsequently built up antiseptic surgery. It was left to a German scientist, Robert Koch, to add a further step which has been of the greatest importance to medical science and industrial chemistry. He showed how it was possible to isolate micro-organisms and grow them separately on culture media. Koch prepared nutrient media with a matrix of gelatin or other suitable substances, which melted on heating but set solid upon cooling. This seems a small detail until its possibilities are appreciated by actual experiment.

Such a solid medium, when sterile, provides a surface upon which a spore or micro-organism may settle; here it remains, until as a result of its growth and multiplication in numbers, it becomes a colony large enough to be seen by the naked eye. Glass dishes, Petri dishes, are constructed with tightly fitting glass lids, so that when the nutrient medium has been poured over the bottom, or plated, as it is termed, the surface of the medium can be examined through the glass. A suitable nutrient medium for many purposes is readily made by obtaining beerwort from a neighbouring brewery, adding to it 10 per cent. gelatin and, when liquid, pouring it into a set of test tubes, which are then plugged with non-absorbent cotton wool. These test tubes are placed in a metal basket, covered lightly with tinfoil and sterilised in the usual manner. The Petri dishes, or other glassware required, are usually sterilised in steam at 128° C. in an autoclave. This higher temperature even kills spores, the form in which any organisms are likely to remain alive for long and so accumulate on glassware; the same method is not suitable for most media as the high temperature is apt to break down the organic constituents. After sterilising, the Petri

dishes are kept wrapped up until required. A tube of beerwort gelatin is taken from a water bath, in which it has been warmed just enough to melt the gelatin, the cotton-wool plug is flamed to kill any organisms that may have come to rest upon it, and to prevent infection from the lip as the liquid contents are quickly poured into a Petri dish, the lid of which is carefully held over the dish during the pouring process, to prevent access of any falling spores. If the operation is carried out in a still atmosphere, practice will enable the operator to carry it out so quickly and dexterously that the sterile fluid will have been poured without opportunity for any spore to fall upon the medium from the air of the room ; it will then remain sterile. A very effective experiment to show that the presence of organisms upon it will subsequently be revealed by their growth, may be carried out by allowing a fly to walk on the medium in the Petri dish. If the fly is then released and the dish placed aside in a cool place, when examined a few days later, the marks left by the contacts of the fly will be seen as little discoloured patches, where various small organisms are increasing in numbers so rapidly as to form visible colonies.

If a few dozen test tubes of sterile beerwort gelatin and the necessary sterile Petri dishes are available, the process of plating may be employed to isolate micro-organisms from one another. For this purpose a small sample of yeast (*Saccharomyces* = sugar fungus) is very suitable. This is the small organism which is so important in the brewing and baking industries, as also in cider making and the production of wine. The test tubes of beerwort gelatin are kept in a water bath and the temperature controlled so that, whilst the gelatin remains liquid, the temperature does not rise above 25° C., and is not sufficiently high to kill the yeast. Whenever in the plating process a test tube is opened, the cotton-wool plug must first be flamed. A tiny speck of yeast is introduced into a tube and well shaken up so that it is fairly uniformly distributed through the fluid ; most of the liquid is then poured into a Petri dish with the usual precautions. A drop is left in the bottom of the test tube and this is poured into a second tube of sterile gelatin and the process repeated. When the second plate has been poured, the residuum in the test tube is again poured into a third tube and in this way

about four successive plates may be poured. In these four plates the original yeast is scattered throughout the beerwort gelatin, now set solid at the bottom of each Petri dish, and in each dish the yeast cells will be at a progressively increasing state of dispersion. The dishes are now set aside, preferably upside down as this prevents drops of condensed liquid from falling on to the surface of the medium, and are examined from time to time. Each separate yeast cell is dispersed in a medium of beerwort gelatin, in which yeast grows very readily, and so, in the course of a few days, from every cell a little colony will develop, about the size of a pin head. Some of these colonies will pile up on the surface of the medium, as yeast grows freely in air; other colonies will develop immersed in the medium, since yeast can also grow anærobically.

In the first plate poured, the colonies will be so numerous that probably, by the time they have each reached the size of a pin head, they will have joined together, but in the next few plates they will be progressively farther apart, and at least one plate will probably have them sufficiently dispersed to allow of examination of single colonies. From any colony cells may be taken for microscopic study, or for continued cultivation if desired. For the latter purpose they should be removed from the plate by a sterile instrument, the most convenient being a piece of stiff platinum wire mounted in a glass rod. This can be heated red-hot just before use, allowed to cool, and then thrust into the colony (care again being taken to hold the lid over the Petri dish during the process), then withdrawn and either plunged into a tube of solid gelatin, or stroked along the surface of a gelatin slant, produced by allowing a tube to cool in a sloping position. In this way stab and streak cultures, respectively, are obtained. The stab culture enables the observer to see whether the organism grows better at the surface (i.e. is ærobic) or beneath it (i.e. is anærobic). In the streak culture the accumulating colony of the organism heaps itself up to give a growth form, often very characteristic of the species.

The procedure, thus briefly described, obviously allows of considerable extension and development, with the use of different media for different organisms, etc. Thus the medical bacteriologist, who wishes to cultivate organisms that have

been growing in the human body, uses an agar medium which is still solid at blood temperatures ; the soil bacteriologist uses media with various inorganic nutrient substances, sometimes using silica gel as the solid matrix.

The yeast organism, thus separated, is worth examination and brief consideration because of its great practical importance, and because it exemplifies the necessity of a knowledge of such organisms and their manipulation in connection with industrial processes. The yeast cells are very small, and the usual cell constituents are less clearly distinguished than in the cells of higher plants. Each small oval cell has a thin wall which encloses the cytoplasm and nucleus (which is not possible to see in the living cell), one or more small vacuoles are also present. As the product of its activity, the cell forms glycogen, a carbohydrate which may be recognised in the cells by the red-brown colour produced on addition of iodine. In a favourable medium the cells multiply rapidly by budding ; an outgrowth appears at one end of the cell, this gradually enlarges and part of the nucleus is separated into it by a process more like a simple constriction than normal nuclear division, the bud increases in size whilst the connecting neck joining it to the parent cell constricts until finally the cells separate. The new cell formed in this way soon reaches the same size as the parent, but even before this it often starts to bud off another daughter cell itself. In this way the cells multiply very rapidly forming simple, or branched chains of cells, and these soon build up a visible colony. Being a saprophytic organism it has to be supplied with organic matter ; supplied with sugars, the yeast breaks these down to alcohol and carbon dioxide, using the energy thus liberated for its own growth. In the presence of oxygen, more of the sugar in the medium is broken down to carbon dioxide, less released as alcohol, and under these conditions the cells multiply more rapidly than when the plant is growing anaerobically. The breaking down of the glucose into alcohol and carbon dioxide is facilitated by the enzyme zymase ; this can be extracted from the cells, but only with great difficulty.

The brewing industry is based on a long chain of reactions controlled by enzymes. In the first place barley is allowed to germinate for a few days, when the starch in the endosperm of the grain will be broken down by the diastase in the grain to

maltose and glucose. At this stage the germinating barley is dried and then ground up and extracted with water; this gives the malt liquor, in which the yeast then converts the glucose into carbon dioxide and alcohol.

In baking, from the sugar formed from the starch in the flour, carbon dioxide is released by the action of yeast, expanding the sticky gluten of the dough and thus making the bread lighter. In the cider and wine industries, sugar from the fruit is fermented and alcohol produced, either through the agency of yeasts, which occur naturally on the skin of the fruit, or are added from pure cultures of specific strains of yeast.

The great importance of these organisms in these processes is largely associated with their extremely rapid rate of growth; from the organism introduced from the skin of the fruit or from the culture, soon many millions are spread through the nutrient, sugar-containing liquid in which it finds itself, and so a most vigorous agent of chemical action is provided, which may be of the greatest value so long as it can be adequately controlled. Thus the chemist has to his hand in the form of these organisms, workmen, who, if the conditions are suitable and controlled, utilise little of the food medium for their own growth, whilst promoting with great vigour and with no request for an eight-hour day, various specific chemical changes. More and more processes, such as the production of vinegar, of intermediates in the dyeing industry, of the chemicals needed for explosives, of glycerine, etc., depend to-day upon such organisms. The need is obvious for their careful study, for an understanding of their life-history, and of the conditions under which they multiply and carry out their metabolic processes. The basic principles for this study are found in the methods for sterilising media and apparatus, for the isolation and growth of the organisms in pure culture.

The yeasts are known to include very numerous species, many of which differ slightly in microscopic features, in the form of the colonies produced in pure culture and the chemical reactions they produce. Most of the species, besides multiplying rapidly by the process of budding, also are known to produce spores under conditions unfavourable to growth. Spore formation can usually be induced in yeast by growing the cells upon sterilised pieces of plaster of Paris, or even better

on slices of carrot. The contents of a cell round off to form one to four spores, each of which has very homogeneous contents. The spores are so small that in unstained material it is difficult to recognise them with certainty, but as the spores have special affinity for certain stains, they may be recognised by the following staining procedure. Smear the yeast as a thin film on a cover-glass and, when nearly dry, immerse in a hot solution of fuchsin (basic fuchsin one part, absolute alcohol ten parts, 5% carbolic acid solution, 100 parts) and warm for ten minutes. Decolorise in 1% sulphuric acid or methylated spirit, which removes the stain from everything but the spores. Wash in water. Stain with saturated aqueous methylene blue for half a minute. Rinse in water, dry and mount in balsam. The spores are stained red, the walls of the original cells blue.

On account of this production of spores inside a parent cell, a process which in some yeasts and many of the related fungi is preceded by a conjugation of cells, yeast is grouped with the Ascomycetes. Many of these are commonly seen in fields and woods, forming cup-shaped or other forms of fruiting bodies bearing cells known as asci, in each of which eight spores are formed.

CHAPTER XXXIII.

THE REPRODUCTIVE PHASE. THE FLOWER. THE STAMEN.

As the normal flowering plant develops a succession of adult leaves there is an increasing proportion of carbohydrates in the supplies reaching the growing apices, and sooner or later new types of structures appear at these growing points. These are the various constituent parts of flowers, and are all either directly or indirectly associated with the production of seed, into which some of the excess food supply will pass, to nourish a new generation of plants at the expense of the parent. Thus the parent plant has entered the reproductive phase.

The Flower.

The advent of the reproductive stage is first evidenced by a decrease in size, and often a change in form, of the leaves, but the flowers that follow are sharply delimited by the different type of structures composing them. It is, however, not easy to give a comprehensive definition of a flower. When the flower is being developed at a growing point, the apex is still producing lateral structures, but usually these are not separated by extended internodes, so that as a first approximation we may define a flower as a short shoot axis, bearing structures associated with reproduction. The condensed region of the shoot axis in the flower is described as the receptacle.

To obtain a clearer conception of a flower we must analyse more closely the nature of the essential reproductive structures. These are found to be of two kinds, the carpel within which the ovules or future seeds are found, and the stamen, the pollen-bearing structure, as the pollen is found to play an essential part in the production of seeds from the ovules. These essential structures are usually accompanied by other

flower parts which play only an indirect part in seed production. The four categories of flower parts which are very commonly present may be well illustrated from a species of the genus *Ranunculus*. The earliest of these to flower in the spring is the lesser celandine (*Ranunculus Ficaria* L.). The flower bud is a rounded structure, and at this stage the inner flower parts are completely enclosed by the three concave green sepals which together constitute the calyx. As the bud opens the sepals become yellower in colour, but are quite distinct from the eight or nine longer petals which form the corolla. The petals are very glossy on the upper side, and at the base of each is a small scale which covers a patch of nectar-secreting tissue. Within the corolla follow the stamens, which are very numerous in *Ranunculus* and indefinite in number; collectively they form the androecium. In the centre of the flower, and towards the apex of the conical receptacle, lie the carpels, which again are several in number and indefinite; these form the gynæceum. Functional flowers must contain either stamens, or carpels, or both, but the term flower is used to cover also certain abnormal forms produced under conditions of cultivation, in which only the inessential parts such as sepals and petals are present, and which therefore take no part in reproduction.

The Tulip Flower.

In a tulip the individual parts of the flower are larger and therefore more suitable for examination. The flower terminates an axis bearing several typical monocotyledonous leaves, which fall off in size as the flower is approached. When the flower bud emerges above the last leaves, the outer parts of the flower are green, but as the bud opens, the outer parts develop the bright colour characteristic of most tulip flowers. These coloured structures overlap one another and completely surround the essential flower parts within (Fig. 123*a*); they are crowded on the axis without extended internodes between their insertions, though they were probably produced at the growing apex in spiral succession like the leaves which preceded them. In tulips the leaves are often arranged in $\frac{1}{3}$ phyllotaxis, in which they fall into three vertical rows and the coloured parts, which are obviously of the nature of modified

leaves, are arranged in two whorls of three, which we could regard as two telescoped turns or cycles of the phyllotaxis spiral. In monocotyledons, whorls of three are common, whilst in dicotyledons, whorls of four or five parts are more usual.

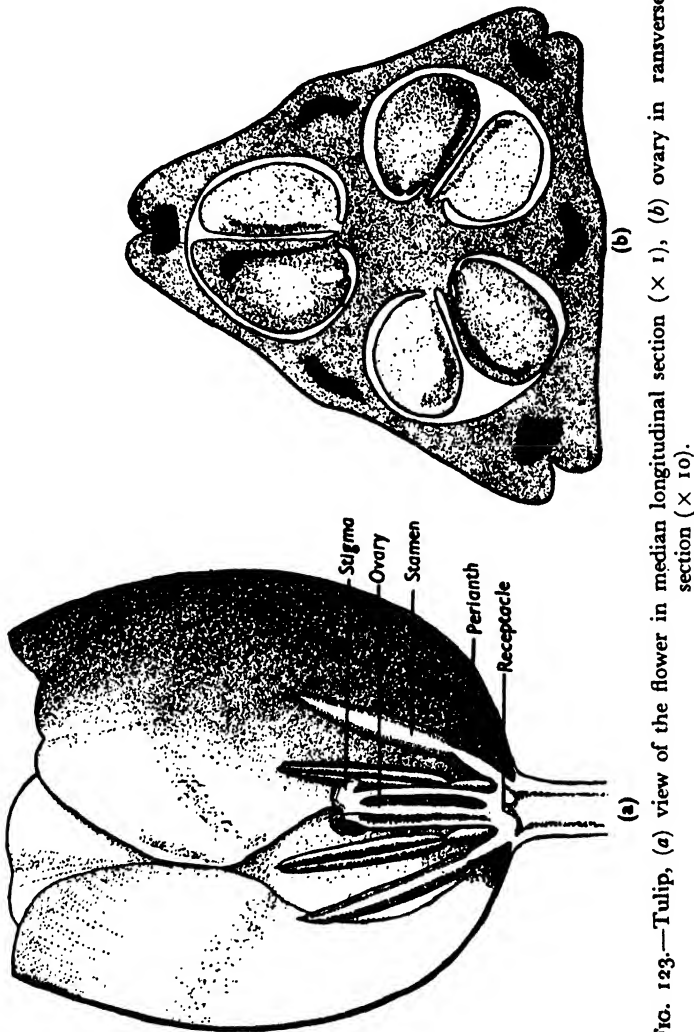


FIG. 123.—Tulip, (a) view of the flower in median longitudinal section ($\times 1$), (b) ovary in transverse section ($\times 10$).

In the tulip no distinction can be drawn between these two outer whorls of flower parts, and they are described as the perianth, consisting in this type of 3 + 3 perianth segments. The segments of the two whorls alternate, and

regular alternation applies also to the remaining whorls of flower parts. Within the perianth are six stamens, also arranged in two whorls of three. Each stamen consists of a stalk or filament, which is continued upwards as the connective between the two long anther lobes. In the open flower the anthers are usually split open lengthwise and are covered with masses of pollen grains, which have been liberated from the pollen sacs in the anthers.

Within the inner whorl of stamens, and forming the central structure of the flower, is the gynæceum. This forms a column, triangular in section, each angle of which terminates above in an outwardly curved lobe with a papillate or almost velvety surface, which is often moist with a sticky secretion, to which pollen grains may adhere. This three-lobed stigma is joined by a narrow neck, or style, to the ovary, which, when cut across transversely, is found to be a three-chambered structure with two rows of ovules in each chamber (Fig. 123*b*). Owing to the shortening of the axis, with consequent crowding together of the flower parts, it is very common to find that the central flower parts, where this crowding effect will be most intense, become fused together in development, and in tulip the triangular outline of the ovary, the presence of three chambers, or loculi, containing ovules, and the three lobes of the stigma, may be interpreted as meaning that three carpels have contributed to its formation. In such a case the gynæceum is described as syncarpous, in other cases the carpels may remain free from one another, as in the apocarpous gynæceum of the buttercup. In either case the essential feature of the angiosperm is present, in that the ovules or future seeds are completely enclosed.

The Stamen.

The structure of the stamen is most easily followed if examined in the flower bud, before the anther has split open to release the pollen. At this stage the filament is relatively short whilst the anther is well developed. On each side of the connective is an elongated anther lobe, each of which has a deep longitudinal groove, which marks the line between the two pollen sacs contained in each anther lobe. The detailed arrangement and structure is seen clearly in a

transverse section of a young anther (Fig. 124). A large proportion of the section is seen to consist of parenchyma-

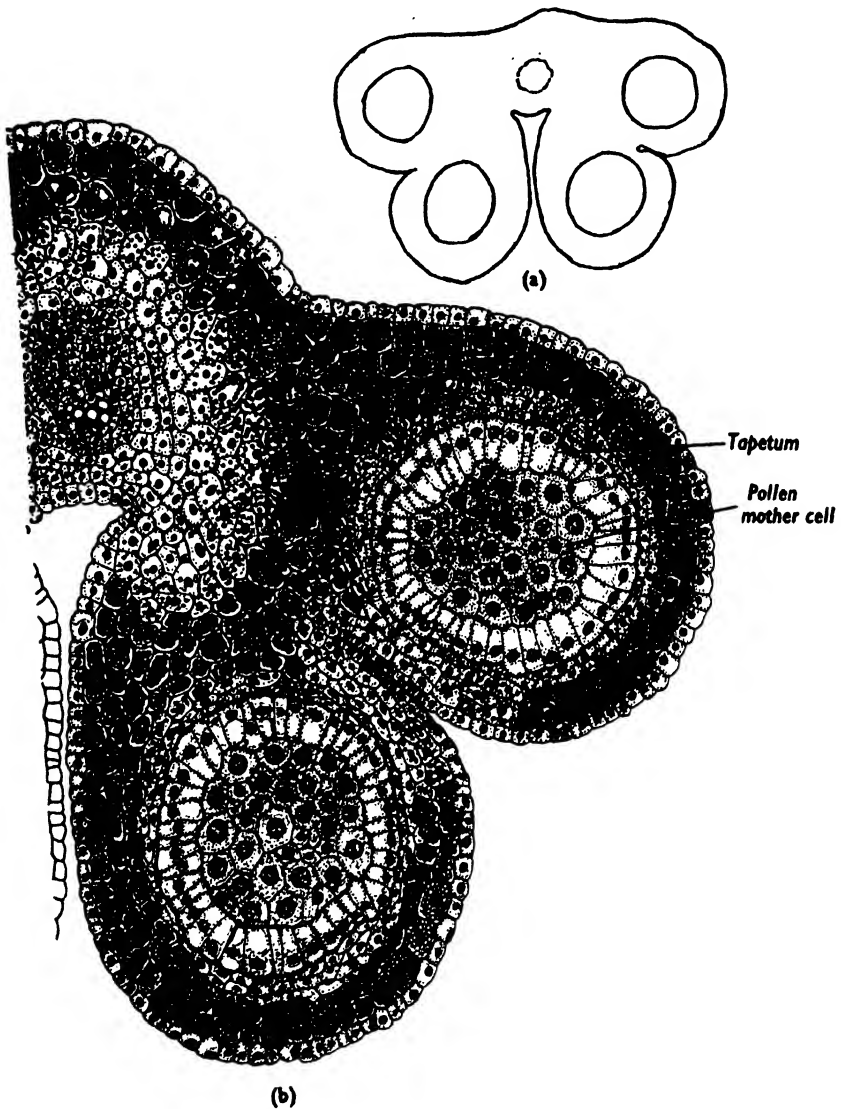


FIG. 124.—Lily, young anther of stamen in transverse section (a) in outline ($\times 25$), (b) half the anther ($\times 85$).

tous cells with protoplasmic contents and conspicuous nuclei. Between the anther lobes lie the tissues of the connective,

containing a small vascular strand. The tissue of each anther lobe is seen to be divided into two circular pollen sacs, the central cells of which stand out very conspicuously in the section owing to their larger size, dense protoplasmic contents, and prominent nuclei. These cells are angular and closely packed together and, though sunk beneath the surface of the tissue, they are evidently able to grow very actively indeed, and have been able to manufacture protoplasm sufficiently fast to keep pace with their growth and multiplication, whilst the cells around have begun to vacuolate and contain starch.

This distribution of the most meristematic cells is perhaps one of the most striking features of the transition to the reproductive phase. It has been seen that during vegetative growth of the shoot, the superficial layers are the most meristematic, and it is to this active superficial growth that the formation of the leaf initials has been ascribed. In the stamen, however, at an early stage of development, the surface cells begin to vacuolate, whilst certain cells lying in a hypodermal position retain dense contents and are conspicuous owing to their larger size. These archesporial cells divide to form the central mass of dense cells characteristic of each pollen sac; eventually they give rise to the pollen grains, or spores, and are consequently described as the sporogenous cells. A somewhat similar development is also found in the carpel, so that the vacuolation of the superficial cells and the continued meristematic growth of more deeply seated cells would appear to be a distinguishing feature of the growth of reproductive organs. With the development of these reproductive structures the growth of the apex is usually brought to a close.

In the section it will be noticed that the walls of the pollen sacs are composed of several layers of cells. The epidermal cells are relatively flat and tabular; immediately beneath them lie large cells packed with starch, and then follow about three layers of smaller cells, flattened parallel to the surface, but lining the wall of the sac is a very striking layer of cells known as the tapetum, the cells of which are elongated at right angles to the surface and are less vacuolated than the rest of the wall cells.

At this stage the anther undergoes a rapid expansion, which is largely due to the final extension of the cells of the wall

as they complete their vacuolation, and the sporogenous cells then no longer completely fill the cavity of the pollen sac as a densely packed mass, but round off from one another as the pollen mother cells, now lying in a liquid medium. These mother cells continue to grow very vigorously, their nuclei undergo two very characteristic divisions in rapid succession, and each division of the nucleus is also followed by the formation of walls between the daughter nuclei, so that there results from each mother cell a group of four cells, the pollen grains. During this process the tapetum and the two or three inner wall layers disorganise, and their contents are added to the liquid surrounding the developing pollen grains.

If an anther is cut across at this stage, the liquid contents of the pollen sacs can be smeared along a clean microscope slide and, with suitable staining, beautiful preparations can be obtained to show all stages in the division of the mother cells to form the pollen grains. This can only be done whilst the contents of the sac are still liquid, a stage which is not prolonged and is passed through at very different times in different plants. In many of the bulb plants such as tulip and hyacinth, it takes place while the flower buds are still enclosed within the bulb, between September and November, the exact time varying with the variety. In the lily, the stage can be found in small flower buds of many species grown in the open during late May or early June, and in the spiderwort (*Tradescantia virginiana* L.), some of the flower buds will probably be found in the right condition during June or a little later. The walls of the mother cells are thin at this stage and suitable stains penetrate quickly.

The rapid growth of the anther, and particularly of the mother cells, each of which gives rise to four pollen grains, each approximately the size of the original mother cell, makes great demands upon the liquid in the sacs, which soon disappears as it is absorbed into the grains. Some of the fatty substances, with other materials, are left as a sticky deposit lining the cavity of the sac and also covering the surface of the grains. In the lily the grains remain for a time in groups of four, and this fatty deposit is laid down most thickly on the surface of the grains which is exposed at this stage, so that when the mature grains separate, the more rounded outer side of each shows this outer coat very prominently; it has then

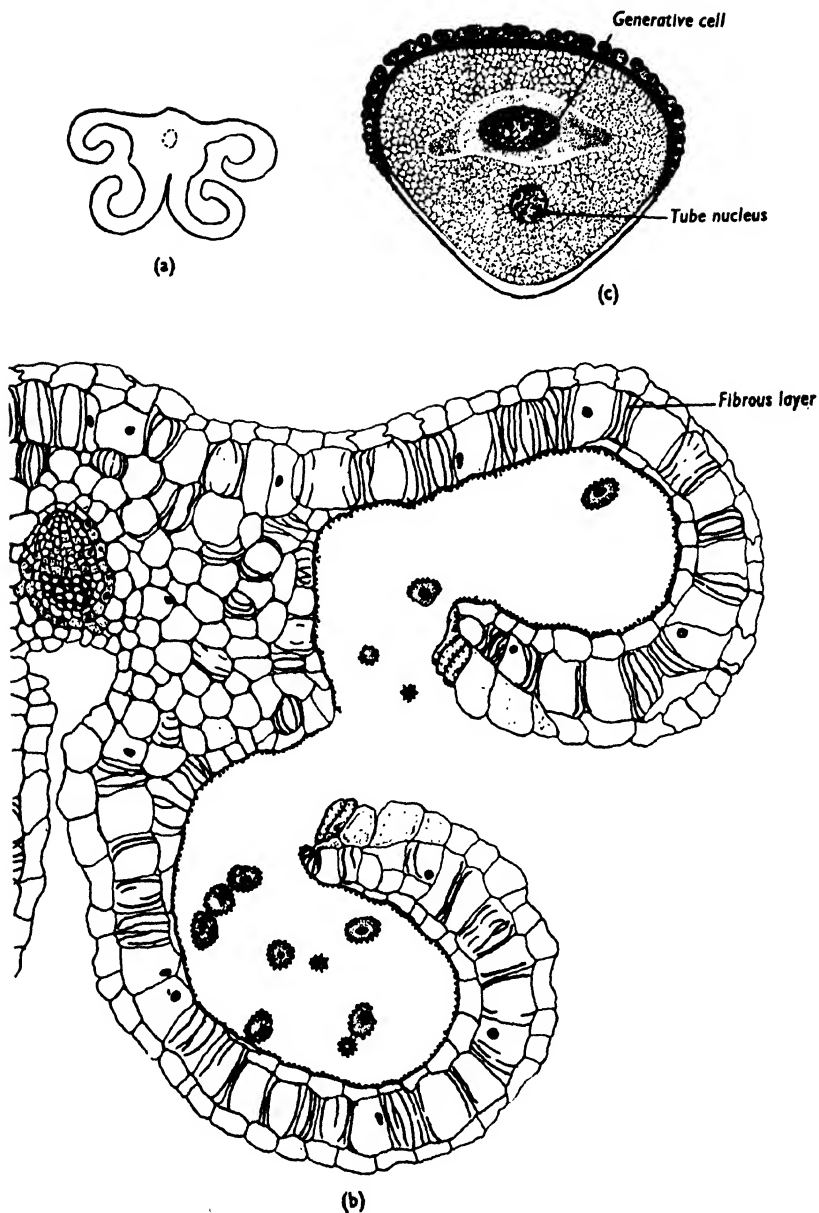


FIG. 125.—Lily, dehiscent anther of stamen in transverse section, (a) in outline ($\times 12$), (b) one half ($\times 75$), (c) single pollen grain ($\times 880$).

dried and contracted into a striking network pattern, whilst the more angular facets of the grain, which lay against the neighbouring grains of the same group, have less of this cuticular coat or extine overlying the smooth inner coat or intine, composed of cellulose and pectin.

Whilst these changes have been taking place in the sporogenous tissue, other changes have been proceeding in the wall layers. Since the disorganisation of the tapetum and inner wall layers, the outer wall of each pollen sac will be about three cell layers in thickness, and of these the sub-epidermal layer consists of especially large and prominent cells, except along the groove between two adjacent pollen

sacs, where it is but little developed. Only a narrow strip of tissue is left between the pollen sacs of the same anther lobe, as this partition consisted largely of the layers which have broken down; this longitudinal line consequently presents a natural position for the subsequent dehiscence of the mature anther. By this time the starch has all disappeared, but a large amount of material has been deposited on the cell

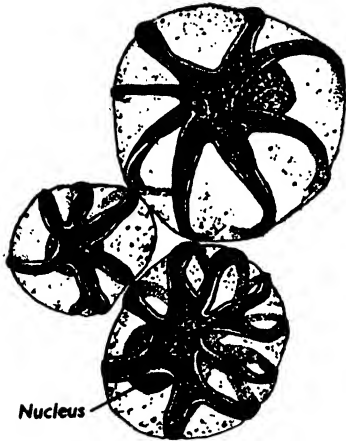


FIG. 126.—Tulip, three cells of the fibrous layer of the anther wall in surface view ($\times 500$).

walls, especially of the large sub-epidermal cells and, on several layers of the tissue between the two anther lobes, in the form of thick, and often lignified, bars; this part of the wall is therefore described as the fibrous layer (Fig. 125). If a piece of the wall of an adult anther, with adhering pollen grains brushed away, is mounted in phloroglucin and hydrochloric acid, it is possible to examine the fibrous layer in surface view, when it is seen that the thickening forms a central thick plate on the inner and outer cell surfaces, whilst arms of thickening run round the sides of the cells linking the plates together. The central plate of thickening is very marked in buttercup anthers, where each cell in surface view has a very striking stellate pattern; in tulip the plate is less evident, and the

pattern consists almost entirely of isolated or radiating bars (Fig. 126).

As the liquid in the pollen sacs is absorbed by the developing grains and the tissues of the anther dry, the individual cells of the anther wall tend to contract and are thrown into a state of strain. On either flank of each anther lobe the fibrous layer of each pollen sac is firmly embedded in the fibrous tissue of the connective, but along the groove between two adjacent sacs, where the wall has largely disorganised and the fibrous layer is interrupted, the wall can stand little strain and soon splits, exposing the pollen of the two adjacent sacs. The pollen grains emerge as glistening masses which reflect the light from their thick waxy coats; sometimes they are yellow, sometimes brightly coloured.

In the majority of stamens the dehiscence is in the form of a long slit, as has been described for tulip or buttercup. The stamen of lily is also a large form convenient for examination, and here a slight difference is found in the relation of the filament to the anther. In the young stamen the anther is erect, but at the time of dehiscence the anther turns at right angles to the filament and it is seen that the latter is only joined to the anther half-way along its length. A cross-section of the young anther below this point, therefore, shows the vascular strand and surrounding tissues of the filament lying between, but free from the tissues of the two lobes of the anther. In this type the mature anther can rock backwards and forwards, pivoting about its attachment to the filament, and is described as versatile. In the heaths (*Erica* spp.) and rhododendrons a different manner of dehiscence is observed, the pollen is liberated through pores developed at the distal ends of the pollen sacs.

The development of the pollen in most dicotyledons differs, in one respect, from the description given above for the anther of tulip, which is typical for the majority of the monocotyledons. When the nucleus of the mother cell undergoes the two divisions in rapid succession to form the pollen grains, the second nuclear division often occurs before the products of the first division have been separated by the deposition of walls. Thus four protoplasmic masses are formed in contact with each other, and they naturally arrange themselves in a tetrahedral manner within the mother cell.

This arrangement is very easily observed in *Rhododendron* or any other flower of the heath family, Ericaceæ, for in these types the pollen remains grouped in tetrads, and is even discharged from the anther in this condition.

To see the true shape of the mature pollen grains in tulip, they must be mounted dry on the slide and examined under the microscope by reflected light, as immediately water is added they swell up, become completely rounded, and often burst. Even in water it is not possible to make observations upon the contents of the grains, for the sculpturing of the extine obscures the view. By special methods, which have previously been described for the preparation of sections of the shoot apex, it is possible to obtain thin stained sections of the mature anther with some of the mature pollen grains still contained within it. Some of the grains are then seen in surface view and show very well the sculpturing of the wall, which varies considerably in different plants. Other grains are cut and some show two nuclei present in the same grain. That this is true of all the grains can also be shown if grains from mature, but undehisced anthers, are mounted in aceto-carmine solution (40 per cent. acetic acid saturated with carmine, whilst boiling) or acetic methyl green, in both of which the nuclei are stained, or if adult grains are mounted in Eau de Javelle, which swells the intine and causes the extine to be shed. Of the two nuclei present in each grain, one is surrounded by a distinct sheath of protoplasm, which is more transparent than that occupying the greater part of the cavity of the grain. The second nucleus has no special protoplasmic sheath (Fig. 125c); when the grains are mounted in water and the contents are forced out by gentle pressure upon the cover glass, the two refractive nuclei can often be recognised, and it is seen that one remains surrounded by its own protoplasm. This nucleus, from its subsequent behaviour, is known as the generative nucleus, or more often the nucleus, together with its attendant protoplasm, is spoken of as the generative cell, though no wall is usually present to delimit it from the rest of the protoplasmic contents of the grain. The other nucleus, lying in the general protoplasm, is the vegetative or tube nucleus.

After the anther has dehisced, the future fate of the pollen is very uncertain, but if seed is to be formed in the fruit, some of the grains must reach the surface of the stigma. The transfer

of pollen from anther to stigma is the process of pollination. In the tulip the rough waxy coats of the grains probably adhere to visiting insects and are later rubbed off when the insects visit other flowers, where some of the grains may be caught on the sticky stigmatic surface. The interplay of floral structure and insect visitors in this process affords a fascinating chapter in biology and will be briefly touched upon later ; for present purposes we may pass on to consider the fate of the grain when this transference has taken place.

On the stigmatic surface the grains come in contact with the sugary secretion from the cells and germinate. They germinate in a very similar manner if they are placed on a glass slide, on a drop of 1.5 per cent. gelatine, dissolved in 10 per cent. cane sugar. The microscope slide is then rested on supports, face downwards, over water or damp cotton wool, and the whole chamber is covered with a glass plate. After some hours, varying with the species of plant and the condition of the pollen, examination under the microscope shows that the grains have swollen, the intine has burst through the extine and has emerged as the pollen tube.

In lily and tulip the tube emerges through the extine where the latter was less developed, where the surface of the grain originally faced the centre of the group formed from one mother cell, but in many kinds of pollen definite thin places or germ pores are present in the wall, and it is very probable that these also owe their origin to contacts between the grains during development. The intine is a very extensible wall, largely composed of pectins, and, as the grain germinates, it is rapidly extended into a long tube into which flow the contents of the grain. As in the growth of a root hair, the apex of the tube remains full of protoplasm, which is closely associated with the region of the wall where extension is taking place ; behind the apex the grain and tube will both be vacuolating, taking in water rapidly, and the expanding vacuole is an indication of the osmotic pressure which is forcing the protoplasm against the apex of the tube and contributing to its continued extension. The growth of the pollen tube is an extremely rapid process, and very commonly the protoplasmic contents can be seen to be moving actively towards the apex. In this protoplasm the nuclei are carried, the tube nucleus passing into the tube first, followed by the generative cell. In the tube the generative

cell undergoes a further division into the two male cells, or male nuclei (since the protoplasmic sheath may or may not persist after this division).

In order to see the germination of pollen grains under natural conditions upon the stigmatic surface, no more suitable plant can be chosen than the common chickweed (*Stellaria media* L.). The flowers do not persist long and are best taken in the afternoon, when fully open, or if taken in the morning, it

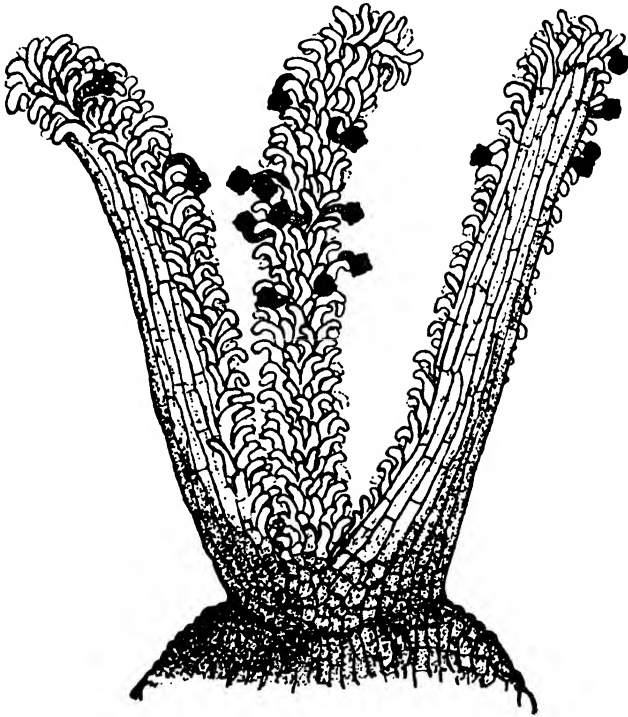


FIG. 127.—*Stellaria media*, stigma with germinating pollen grains ($\times 115$).

is necessary to select the closed flowers which were fully open the previous day. In the centre of the flower the green ovary is surmounted by three spreading stigmas, which can be removed, still attached to the top of the ovary wall, and placed in a drop of aqueous methylene blue solution. After about a minute the dye is washed off and the stigmas are mounted in a drop of clean water under a cover-glass. The stigmatic surfaces are strongly papillate and often give the violet pectin reaction with the methylene blue. The walls of the pollen

grains stain bright blue and possess distinct germ pores, but where the grains have germinated, the violet staining pollen tube will be seen emerging from the grain and making its way in amongst the cells of the stigmatic surface. Very often a considerable number of grains are seen germinating on the same stigma (Fig. 127). To follow their course down through the loose tissues of the stigma and style is more difficult, but the tubes can be picked up again, in other preparations, as they enter the cavity of the ovary. During this growth, which may be very considerable in flowers with a long style, such as lily or maize, the tubes feed upon the sugary secretions from the cells of the stigma and style.

The further history of the contents of the tube must now be deferred until the development of the carpellary structure has been described, but since the pollen tubes can be traced to the ovary cavity, and thence to the ovules, it is particularly to the ovules, or future seeds that attention must be directed.

CHAPTER XXXIV.

THE CARPEL AND FERTILISATION.

It is the characteristic feature of the angiosperms that the ovules, and subsequently the seeds developed from them, are borne within carpellary structures. A suitable type in which to study the carpel is the marsh marigold (*Caltha palustris* L.). The perianth of the flower consists of a somewhat variable number of yellow segments, which are interpreted as sepals as a result of comparison with allied types. These are followed by numerous stamens, which are spirally arranged around the convex receptacle; their spiral succession is clearly indicated by the way in which the outer stamens mature and shed their pollen first, and then progressively those nearer the centre. In the centre of the flower lie the carpels, which likewise are variable in number, but usually about seven. In Fig. 128*a* it is seen that the carpel has a straight ventral face towards the centre of the flower and a curved dorsal or outer side. The ovary forms the greater part of the carpel and is surmounted by the stigmatic surface, which is roughly papillate and curves outwards from the centre of the flower. A transverse section of the ovary shows a vascular bundle in the dorsal ridge and two in the ventral region. The latter lie in the thickened margins, which only make contact with one another through short proliferations (Fig. 128*b*). The ovules are attached along the length of the carpel in two rows, as may be seen if carpels are split open along the dorsal margin, or if they are cleared in Eau de Javelle. As the ovules are attached alternately to either margin, two ovules are never cut medianly in the same transverse section.

A carpel of *Caltha* arises on the flank of the receptacle as a hemispherical papilla, which grows in length and has an appearance very similar to a young leaf primordium. Soon it becomes concave, and as growth in length continues, the

margins curve inwards and meet and the suture is closed by the proliferation of the surface cells.

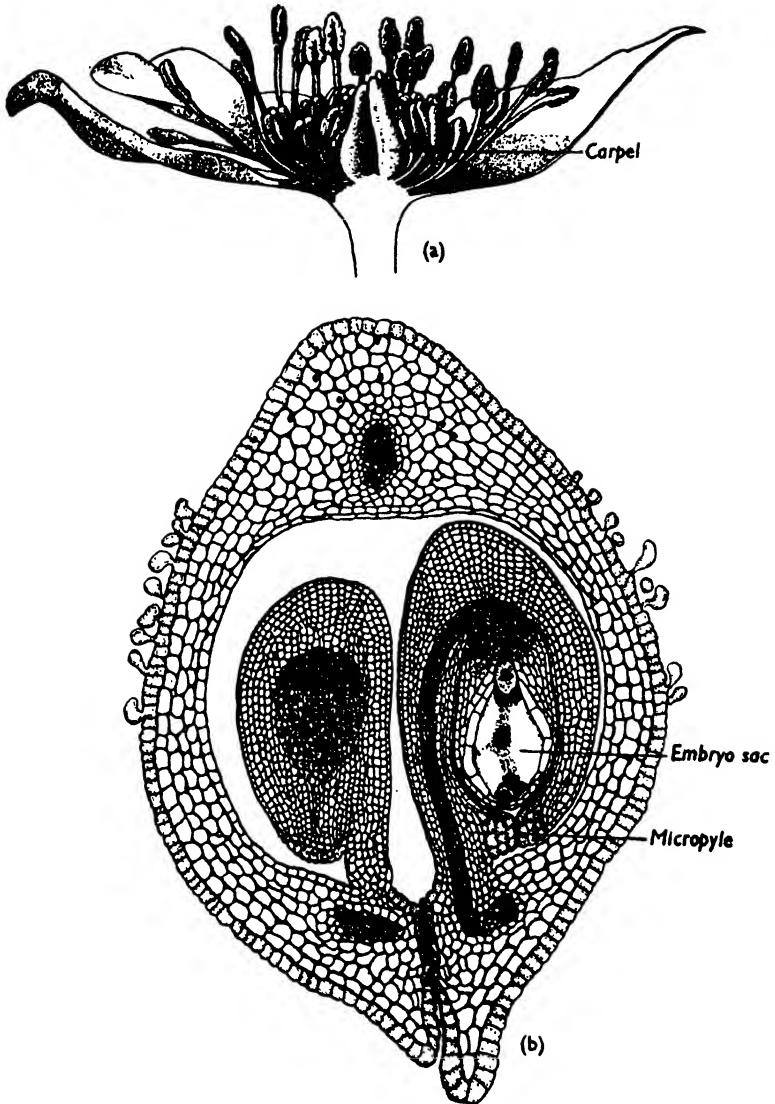


FIG. 128.—*Caltha*, (a) view of the flower in median longitudinal section ($\times 3$), (b) carpel in transverse section ($\times 60$).

In *Caltha* the carpels are so placed on the receptacle that the inner, adaxial surface bears the ovules in a row along each

side of the ventral suture, this manner of bearing the ovules, the placentation, is described as marginal or sutural. If carpels of this form became fused with one another, during development, by their inner faces, then three such fused carpels would give a syncarpous gynæceum of the tulip type (Fig. 123*b*). The ventral margins of the carpels are joined in the centre of the ovary, which appears trilocular, with two rows of ovules in each loculus, attached to the central, or axile, placenta. But when carpel development begins the dorsal side emerges first and the ventral margins are rather later to develop, and in some types the fusion appears to have taken place so early that the carpels have become joined together before the ventral margins of the individual carpels have curved round to meet one another. As a result the ovary has but a single loculus, on the wall of which, at points midway between the dorsal sutures, the contiguous ventral margins of adjacent carpels project into the cavity. The ovules are localised on these regions of the common loculus, and the placentation is described as parietal.

This method of regarding the carpels, as much modified foliar structures bearing marginal ovules, meets difficulties when the attempt is made to apply it widely to the varied types of gynæceum encountered among angiosperms. This may be illustrated by reference to the families Cruciferæ, Caryophyllaceæ and Primulaceæ. In the wallflower, as in other members of the Cruciferæ, the ovary appears to be constructed of two carpels, which seems probable as the other series of flower parts are borne in whorls of two or four (see Chapter XXXIX). In transverse section of the ovary, it is evident that the ovules are borne on two parietal placentæ, but joining these a partition, the replum, has developed across the ovary, which is therefore bi-locular (Fig. 170). On the usual view that the two carpellary structures have fused at an early stage by their open ventral margins, one would not expect this partition. The replum is therefore sometimes described as "spurious," but this seems an inappropriate term to apply to a constant feature of the ovary, which persists throughout its development and is conspicuous in the fruit.

The ovaries of the families Primulaceæ and Caryophyllaceæ present yet a further difficulty. A transverse section of the ovary shows a single loculus, in which the ovules arise from a

central column which shows no attachments to the wall in this view, a type of placentation that has been described as free central (Fig. 129*a*). In longitudinal section, the central placenta can be seen to rise from the floor of the ovary. In the Caryophyllaceæ it is continuous with the top of the ovary wall, when it is known as partly free central, whilst in the Primulaceæ it is completely detached, though in *Primula* and some other genera, a long shaft runs upwards into the hollow style (Fig. 129*b*). In such a type the pollen tubes, growing down the tubular style, naturally pursue their course on to the surface of this

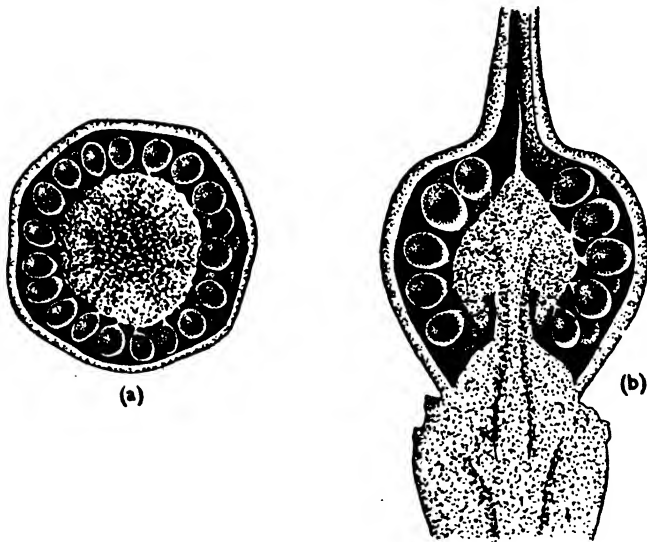


FIG. 129.—Primrose ovary ($\times 16$), (a) in transverse section, (b) in longitudinal section.

slender shaft and thus reach the placenta and the micropyles of the curved ovules.

In the Caryophyllaceæ and Primulaceæ the numbers of floral parts in the outer whorls justify the assumption that the ovary might be expected to arise from several members, possibly as many as five, and in the Caryophyllaceæ the number of free styles suggests that three to five carpellary members are present. The suggestion has therefore been made that the ovary represents such carpels, fused along their ventral faces, and that the partitions, which should have divided up the ovary into loculi, have failed to develop. The ventral margins, with

the ventral suture, are supposed to be fused into the central column. Many assumptions have thus to be made in order to bring such cases into line with the view that the ovary is built up of a number of fused carpellary structures of foliar origin.

In *Caltha* numerous ovules are attached along the marginal placenta of each carpel, but in certain related genera of the same family Ranunculaceæ the carpels are shorter structures, and only a single ovule develops in each. In *Ranunculus* the single ovule arises so low on the ventral suture that it appears to grow from the floor of the loculus, and is described as basal,

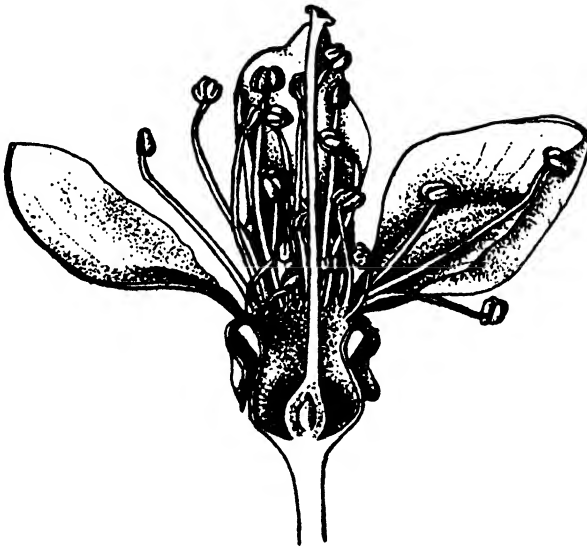


FIG. 130.—Cherry, view of the flower in median longitudinal section ($\times 3$).

whilst in *Anemone* it arises higher and is pendulous from the roof of the loculus. Though in these cases the ovule may be regarded as arising from the wall of the carpel as in *Caltha*, the ovule has clearly a different position of origin in the ovaries of such plants as *Rumex* and *Polygonum*.

In *Rumex*, the flower bud arises in the axil of a bract as a conical upgrowth very similar to the apex of a vegetative shoot (Fig. 132). As development proceeds this apex itself becomes the single ovule, around which the ovary wall makes its appearance at three separate points, which become confluent below and grow up as a cup around the single terminal ovule.

As the ovary wall has three distinct points of origin it is interpreted as consisting of three carpels, which share a single ovule between them. Here the ovule is formed from the apex of the growing bud, and arises earlier than the carpellary wall which finally surrounds it, so that the placenta seems to be part of the receptacle.

In the examples considered so far, the ovary has been situated distinctly higher on the receptacle than the remaining whorls of flower parts, when the flower is described as

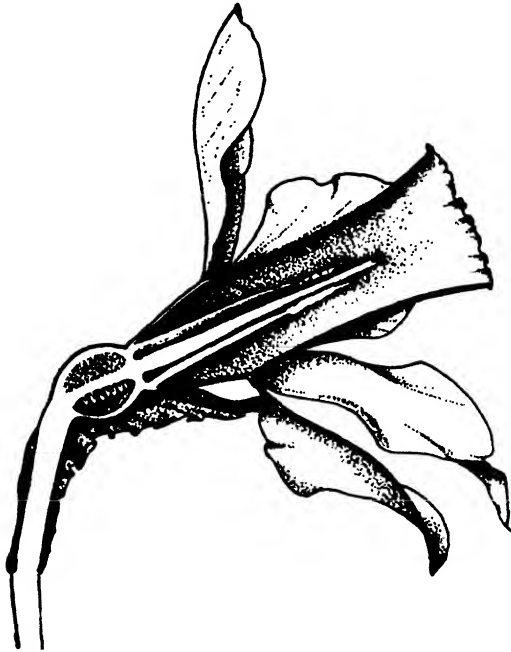


FIG. 131.—Daffodil, view of the flower in median longitudinal section ($\times 1$).

hypogynous and the ovary as superior. In plants belonging to many other families, the receptacle may show all degrees of flattening and in many cases is hollowed out into a cup, from the floor of which arise the one or more carpels (Fig. 130), and from the rim the sepals, petals, and stamens (perigynous). In more extreme forms, such as daffodil, the other flower parts appear to be growing off the top of the ovary (epigynous), which lies completely below them and is described as inferior (Fig. 131). The tissue, which surrounds the loculi containing the ovules,

may be regarded as built up of the walls of the carpels, fused with a deeply concave receptacle. In daffodil there are three

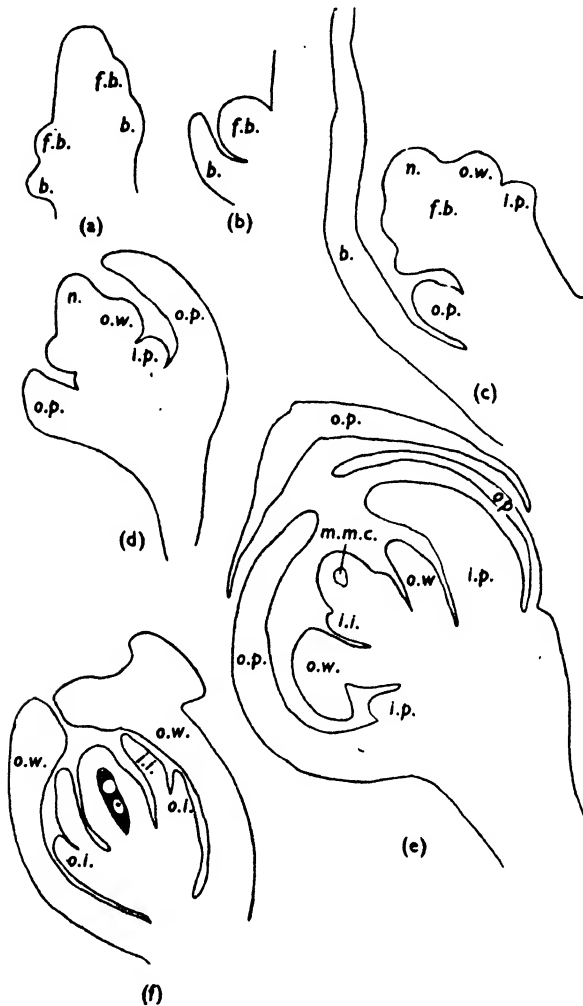


FIG. 132.—*Rumex Acetosella*, stages in the development of the flower ($\times 160$), *b.* bract, *f.b.* flower bud, *o.w.* ovary wall, *i.p.* inner perianth segment, *o.p.* outer perianth segment, *n.* nucellus, *m.m.c.* megaspore mother cell, *i.i.* inner integument, *o.i.* outer integument.

loculi and the ovules are borne upon an axile placenta, as in tulip.

The ovules usually have the same essential features, but

the actual form of the ovule varies according to whether it is borne in a symmetrical position at the end of the axis (*Rumex*), or from the side of a placenta, as in types with marginal or axile placentation. In *Rumex*, a section of a very young flower bud shows the flower developing in the axil of a bract. It is terminated by the nucellus of the ovule, whilst below it may be seen the first appearance of the lobes which will grow up around it as the cup-like ovary wall, and below this again the early stages in development of the segments of the inner and outer perianth (Fig. 132). The stamens are borne in different flowers. As progressively older buds are examined, it is evident that the ovary wall grows up and encloses the ovule, except for a slight aperture at the distal end. Towards this end also, the outer side of each ovary lobe develops an outgrowth, which later becomes crowned with a bunch of long, stigmatic outgrowths. Whilst the ovary wall is developing, there grow up around the nucellus the two integuments, first the inner and then the outer. The inner grows up beyond the apex of the nucellus into a short neck, the micropyle. The outer integument always remains shorter and reaches to the shoulders of the inner.

By the time that the integuments are appearing, characteristic changes also begin to take place in the conical mass of nucleated cells, forming the nucellus. The details which follow are taken from Strasburger's account of development in *Polygonum divaricatum* L., a plant belonging to the same family as *Rumex*. Just below the apex of the nucellus, a single denser sub-epidermal cell divides into two, an upper tapetal cell and an inner and lower archesporial cell. The archesporial cell undergoes two divisions in rapid succession so that a file of four cells is formed. These two divisions, though giving a file instead of a tetrad, are regarded as comparable with the divisions of the pollen mother cell into the four pollen grains, and the parent cell is often described as the megaspore mother cell, though in this case only one of the resulting cells forms a fertile "spore." This cell grows very large indeed, mainly at the expense of the neighbouring cells, which are disorganised and absorbed during its development (Fig. 133).

This cell, in view of its place in the life-history when compared with other types (Chapter XXXVI), is spoken of as a

spore, on account of its large size as a megaspore, although it never lies loose in a cavity, and therefore never develops a thick outer wall, as do the pollen grains and the other spores to be considered later. From its future history in the flowering plants, it is more commonly spoken of as the embryo sac. The rapid increase in size of this cell is due partly to accumulation of protoplasm and partly to vacuolation. During this time the nucleus divides into two, which separate, and then undergo two further divisions so that a group of four nuclei is present at each end of the cell; these eight nuclei

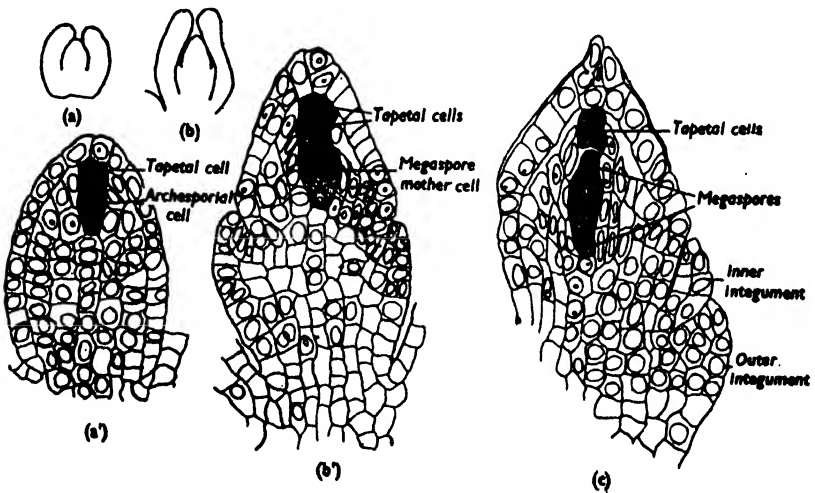


FIG. 133.—*Polygonum divaricatum*, stages in the development of ovary and ovule (after Strasburger). (a) Nucellus in ovary wall ($\times 50$), (a¹) nucellus at the same stage ($\times 270$), (b) inner integument appearing ($\times 50$), (b¹) nucellus at the same stage ($\times 270$), (c) nucellus at later stage, outer integument appearing ($\times 270$).

finally become distributed as seen in Fig. 134, three at the end nearest the micropyle, known as the two synergidæ and the ovum, two (derived one from each polar group) which fuse sooner or later in the centre of the cell to form the definitive nucleus, and three clustered at the other end known as the antipodal nuclei. The two synergidæ and the three antipodals sometimes become surrounded by a distinct sheath of protoplasm, and sometimes even a wall, when they may be described as cells, but the ovum and the definitive nucleus usually lie in the general cytoplasm of the sac. At this stage

the growth of the large embryo sac has so stretched and squashed the distal cells of the nucellus that they disorganise, and the embryo sac is exposed at the base of the micropyle.

In the development of the carpel of *Caltha* the dorsal ridge appears first, and the ventral margins, which are later to

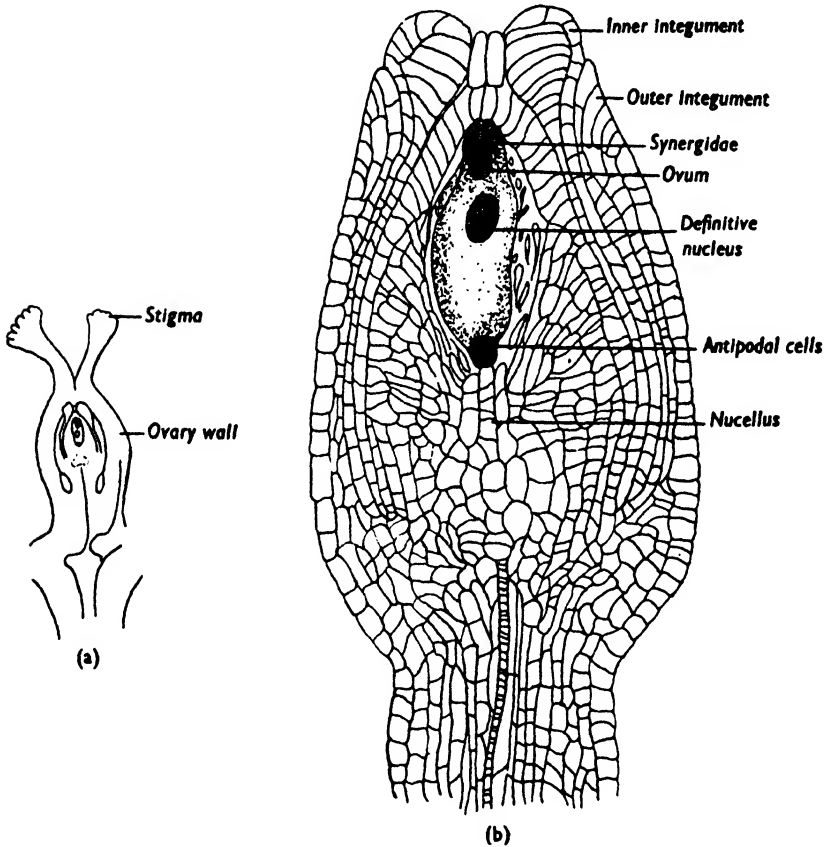


FIG. 134.—*Polygonum divaricatum*, the mature ovule and embryo sac (after Strasburger). (a) The ovule in the ovary ($\times 20$), (b) the ovule at the same stage ($\times 180$).

develop, curve round until they come in contact with one another, and the two surfaces are held together by the proliferating surface cells. Distally, a continuation of this same proliferating surface is exposed as the papillate surface of the stigma. The condition is very similar in the syncarpous types with axile placentation, such as tulip. The joined

ventral sutures of the carpels form a loose central tract, continuous above into the three stigmatic lobes. When the pollen germinates on the stigmatic surface, the pollen tubes probably feed upon the sugary secretions from the surface cells, and then very naturally follow the tract where very similar cells occupy the centre of the style, where such is present, or grow down along the marginal suture in the *Caltha* type. On reaching the ovary cavity the pollen tubes grow out among the crowded ovules and, as at this early stage the micropyle is probably full of liquid contents from the crushed nucellar cells, as they reach its vicinity the pollen tubes grow down the micropyle. When the tip of the pollen tube reaches the surface of the embryo sac, either the liquid in which they lie contains digestive enzymes or, as the result of the mutual pressure of these cells upon one another, the stretched and weakened walls burst and the contents of the tip of the pollen tube are forced into the embryo sac, carrying with them the two male nuclei. These nuclei thus released into the embryo sac ultimately fuse, one with the ovum and one with the definitive nucleus. These nuclear fusions constitute the act of fertilisation; the fertilised ovum becomes enclosed in a wall and is known as the oospore, whilst the other fusion nucleus, really the product of a triple fusion, gives rise to the endosperm.

The developmental stages, which have been described for the ovule of *Rumex* or *Polygonum*, are very uniform throughout the group of flowering plants, but have been taken with particular reference to this type since the ovule, developing from the floor of the ovary, grows into a symmetrical structure. Young ovaries of *Rumex Acetosa* L., cleared in Eau de Javelle, provide preparations in which the basal ovule with its integuments may be recognised. This type of straight (atropous or orthotropous) ovule is, however, relatively rare, and in the great majority of flowering plants the ovules are developed as lateral outgrowths from the wall of the ovary, as in the marginal placentation of *Caltha* or the axile placentation of tulip. In all such cases the growth of the developing ovule is asymmetric. In the commonest type of all, represented by *Caltha* or tulip (Fig. 135a), the ovules commence their growth in the manner described for *Rumex*, but then one side of the emerging meristematic mass makes so much more rapid growth that the

nucellus becomes thrust over until it is completely inverted, so that the apex of the nucellus and the micropyle finally face the surface from which the ovule is growing. The funicle still joins the ovule at the side nearest the placenta, but a continuation of it, including the vascular bundle, runs up fused with the side of the inverted ovule to the nucellar base, the chalaza, and is known as the raphe. In this anatropous type the nucellus remains straight, but is completely inverted. In many other flower types such as shepherd's purse (*Capsella Bursa-pastoris* L.), the nucellus and the included embryo sac have been sharply curved by their asymmetric growth, the funicle appears to join the ovule at one side, and the micropyle

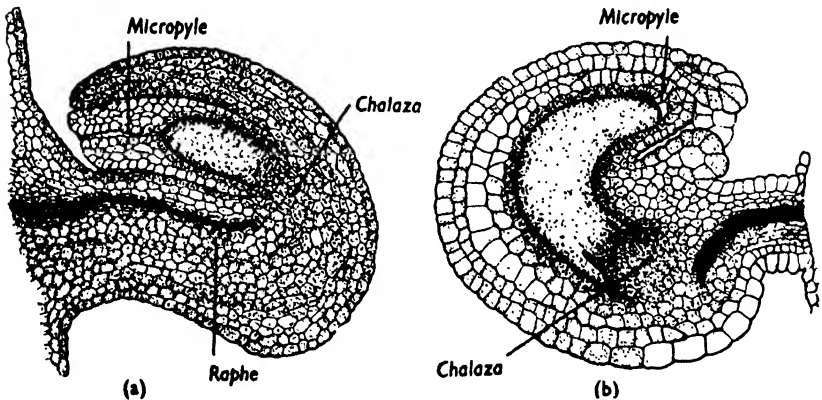


FIG. 135.—(a) Tulip, anatropous ovule ($\times 60$), (b) *Capsella*, campylotropous ovule ($\times 200$).

is again brought round to face the attachment, this type of ovule is distinguished as campylotropous (Fig. 135*b*). As the curvature appears very early in anatropous ovules the outer integument is often suppressed on the side between the nucellus and the raphe.

If young ovules are carefully removed from the ovary of a flower which has been open long enough to be fertilised, and if these are mounted in Eau de Javelle, it is very often possible to see pollen tubes entering the micropyles of the ovules. A particularly good plant for this purpose is *Veronica serpyllifolia* L. (Fig. 136).

The embryo sac cell is so large that it is usually not possible to see all the eight nuclei of the sac in it in a section, but in cross-sections of the ovary of *Lilium* the various nuclei may be

identified by their characteristic positions. If fertilisation has taken place, the male nuclei can be distinguished by their more worm-like form, but it is not often that this exact stage is seen, in which the male nuclei are in the embryo sac but have not yet fused with the ovum and definitive nucleus respectively. After these nuclear fusions have taken place, the two fusion nuclei are the only ones which take part in the further development. Sometimes the definitive nucleus, with some of the

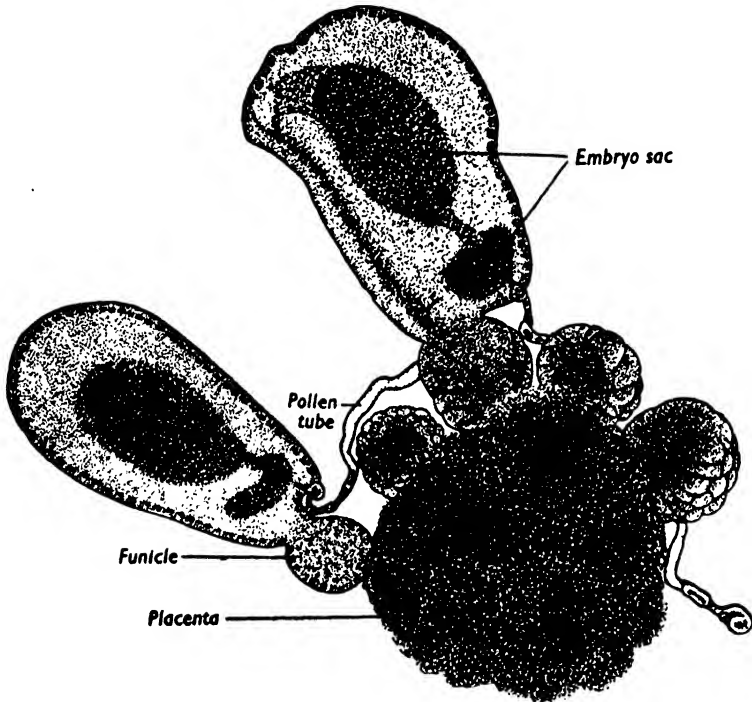


FIG. 136.—*Veronica serpyllifolia*, pollen tubes entering ovules ($\times 65$).

cytoplasm, becomes surrounded by a cell wall, but more often this nucleus divides repeatedly in the general cytoplasm of the embryo sac, when, owing to the presence of a central vacuole, the resulting nuclei lie around the periphery of the cell. As growth proceeds these peripheral nuclei may be separated by walls, thus forming a tissue known as the endosperm. During this phase the nucellus and the integuments continue to increase in size, receiving supplies through the vascular bundle of the funicle; still more rapid is the growth

of the enlarging embryo sac, which gradually crushes the surrounding nucellar tissue, the contents lost from these compressed tissues being absorbed into the developing endosperm.

The oospore is usually later than the endosperm nucleus in beginning to divide, but the degree to which the endosperm may be formed before the oospore commences activity, differs greatly in different plants. The oospore lies near the tip of the embryo sac, at the end towards the micropyle, and its first divisions, in both monocotyledon and dicotyledon, are most interesting and indicative of the differences in types of cell division which will be maintained throughout the life of the new plant. The first division wall in the oospore will be transverse to the long axis of the embryo sac and, of the products of this division, the cell nearest to the micropyle will vacuolate and increase enormously in size, but usually it will not divide again. The deeper lying cell will divide again, producing, next to the vacuolated basal cell, by a series of transverse divisions, a chain of cells known as the suspensor. At the lower end of this chain is found a meristematic cell, the embryo cell proper, which continues to divide by walls in different planes and ultimately gives rise to the embryo (Fig. 137). Comparing these cell types with those present at the shoot apex, it is interesting to find the same sequence :

- | | | |
|--|--|--------------|
| (i) vacuolated cell, | adult parenchyma cell, | basal cell. |
| (ii) vacuolating and
dividing cell, | parenchyma file in
young internode, | suspensor. |
| (iii) eu-meristematic cell, | cell of apical meristem, | embryo cell. |

The further divisions in the embryo cell follow a different path in monocotyledon and dicotyledon. The dicotyledon type is readily followed in *Capsella*, where a number of ovules are present in each ovary and a whole series of flowers, in different stages, is present along the axis of the flowering shoot. In this plant the ovary is two-chambered, and the campylotropous ovules are borne laterally upon parietal placentæ on the wall of the ovary cavity. The endosperm nucleus divides but a walled tissue is not formed. If ovules are scraped off the placenta and mounted in Eau de Javelle, gentle tapping on the cover-glass bursts the large embryo sac cell and the liquid contents emerge through the micropyle, carrying with them the endosperm nuclei and the developing embryo. In the young ovaries, from flowers from which the petals have only

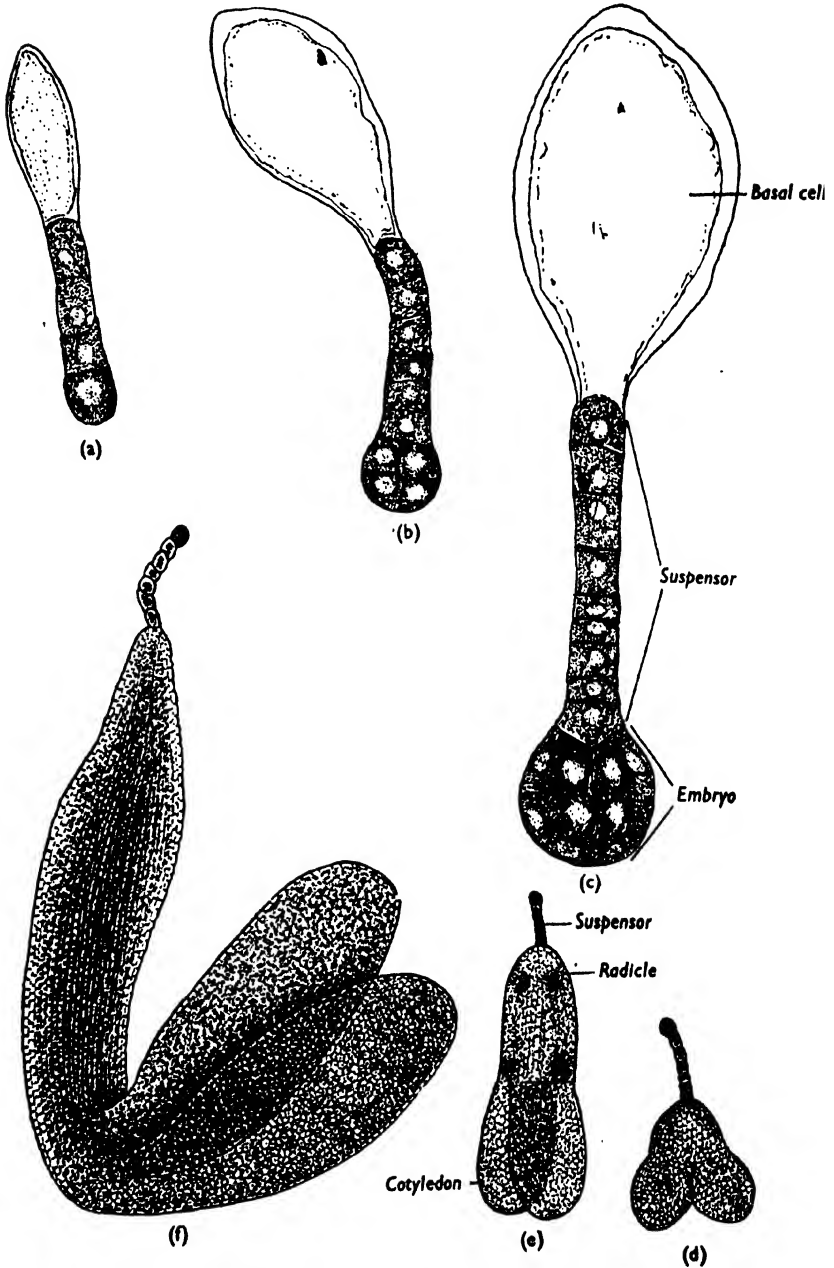


FIG. 137.—*Capsella*, stages in embryo development (a), (b) and (c) $\times 240$, (d), (e) and (f) $\times 50$.

recently fallen, the stage may be obtained where the oospore has given rise to the large basal cell, a chain of about five to seven suspensor cells, and the single embryo cell, but as a series of older ovaries are treated similarly, successive stages in the development of the embryo may be followed (Fig. 137). At first the embryo simply increases in size as a spherical structure, in which the first transverse division is followed by divisions in planes at right angles to one another, which in turn divide the original cell into halves, quarters, and octants. Then, by periclinal divisions, a superficial layer is cut off, which will subsequently maintain itself at the surface in that part of the embryo which gives rise to the shoot, as all subsequent divisions in it will be anticlinal (cf. the dermatogen of the shoot apex, Chapter XV).

As divisions continue and the mass of cells increases, some of the inner cells begin to vacuolate, and the embryo then shows a tendency to elongate in the direction of the long axis of the sac. Immediately this happens, two lobes begin to appear at the end of the embryo distal from the suspensor. These lobes continue to develop until they are recognisable as the first two leaves or cotyledons, and their formation is evidence that surface growth is active at this end of the embryonic structure, the axis of which is now extending in length. At this stage the meristematic area between the folds is practically indistinguishable, but must represent the position of the rudimentary plumule. At the other end of the embryo the meristematic tissue soon begins to show a tendency to divide repeatedly by transverse walls, with the result that gradually a radicle becomes visible which, by its continued extension, pushes the cotyledons further down into the embryo sac. In this plant the funiculus during the enlarging embryo sac is soon utilised by the growing embryo.

Owing to the curvature of the embryo sac in this campylotropous ovule, as the embryo reaches a certain size it also has to become curved, to conform to the shape of the enclosing cell, and thus the cotyledons come to lie over one another, with the one on the outer side a little larger than the other (Fig. 137). The growth of the embryo continues until it completely fills the entire embryo sac, the original contents of which, including the endosperm, have all been absorbed into it. The whole structure, developed from the fertilised

ovum, consists of the massive embryo, which even develops chlorophyll. It lies within the nucellus, which is now a thin compressed layer of tissue, and is surrounded by a firm dry skin, derived originally from the integuments of the ovule, and now recognised as the testa of the seed.

If fertilisation fails to take place, the ovum does not make any further growth and, normally, the whole ovule shrivels away. If fertilisation takes place, not only does the fusion stimulate activity on the part of the endosperm nucleus and lead to the development of the embryo from the oospore, but the whole ovule increases enormously in size to accommodate the growing embryo within. A similar effect is also seen in the surrounding tissues, where the ovary containing fertilised ovules grows rapidly in size to become the wall or pericarp of the fruit and, in many types, the surrounding tissues of the axis, and even other floral parts, may also be stimulated to rapid growth.

In *Capsella* a subsequent hardening of the testa and pericarp takes place, associated with a gradual loss of water from the tissues of the maturing fruit. As the water supply diminishes, naturally movement of soluble food must stop also, and all processes of growth are affected. Thus the embryo gradually passes into a condition of suspended activity in which its tissues, still potentially meristematic, have their growth arrested until abundant supplies of water again reach them. The dry integuments form a firm, hard testa, and as the drying process continues, tensions arise in the complex layers of the fruit wall, which finally lead to a sudden dehiscence of the fruit, whereby the seeds are scattered. Some such process of dehiscence will happen in an indehiscent fruit which contains many seeds, and some of the latter are likely to be distributed by natural agencies to positions favourable to germination.

In some types of developing seeds, fertilisation is followed by a much more vigorous development of the endosperm, which absorbs food from the surrounding tissue and forms a firm tissue filling the embryo sac. The oospore commences growth considerably later and often, when the seed matures, the embryo may occupy only a small proportion of the cavity of the sac. This type of development will give rise to an endospermous seed, in which the food reserves absorbed into the sac are mainly used by the embryo at germination.

In the monocotyledon ovule, after fertilisation, the processes are very similar. A spherical, meristematic embryo cell continues growth at the end of a suspensor, which carries it forward into the developing endosperm. But as the embryo continues to develop, the characteristic monocotyledon habit may be recognised at an early stage. The mass of meristematic cells soon passes from the spherical to the elongate form, and it is possible to see that this elongated mass is only forming one massive lobe, the first leaf or cotyledon. It is only upon germination that it becomes clear that this is not a true terminal lobe but an encircling fold, which grows so rapidly compared with the shoot apex, that the latter appears to be buried within it. The appearance of the embryo of *Alisma Plantago* L., within a mature seed still enclosed in the fruit, was illustrated in Fig. 13. As in the case of dicotyledons, the food absorbed into the embryo sac, in monocotyledonous seeds, may pass almost directly into the embryo, when a non-endospermous seed is formed, or the endosperm may be retained in the mature seed.

In the present and preceding chapters, the essential floral parts, stamen and carpel, have been briefly described, and the rôle of pollen grain and ovule, in the formation of the embryo within the seed, have been examined. It will be seen that the whole of the new plant, which will emerge from the seed, was at one time contained within one single cell, the oospore, and that this cell is itself the product of the fusion of two cells, or at least of two nuclei. Specially formed reproductive structures, and a remarkable train of related events, seem to be involved in the process of fertilisation and the formation of the seed. Such complicated processes are likely to have a long evolutionary history, and it will be our business in the next chapters to try and interpret the meaning of this long series of events and the nature of the structures involved. This necessitates two distinct lines of discussion ; in the first place, the structures are best understood by a comparison of life cycles and reproductive structures in more simply organised plants ; in the second place, the whole process culminates in the fusion of two nuclei, and to understand this, it will be necessary to look more closely into the structure and organisation of the nucleus, and into the processes of nuclear growth and division.

CHAPTER XXXV.

LIFE CYCLES THROUGHOUT THE PLANT KINGDOM.

I. NON-VASCULAR PLANTS. ALGÆ AND MOSSES.

Algæ.

In Chapter XIV we saw that an individual plant might consist of a single cell, as in the unicellular alga *Chlamydomonas*. This particular type was motile, and growth and division of the protoplast was naturally followed by separation of the products of division, so that it was clear that the individual had multiplied, and that its life cycle, as a single individual, was completed by the process of cell division. When the contents of the *Chlamydomonas* cell divide, they first contract away from the cellulose envelope of the mother cell, which ceases to swim about as there is no longer any protoplasmic machinery to keep the flagella in motion. Each cleavage of the protoplast is preceded by the division of the nucleus, which may divide only once, but more frequently each daughter nucleus divides again and the process may be repeated several times, so that the number of uninucleate protoplasts produced within the parent cell is always some multiple of two. The size of the daughter protoplasts will vary according to the number formed. They may develop flagella whilst still within the wall of the parent cell, and the wall finally disorganises when they are released as motile masses of protoplasm, each of which is provided with a chloroplast, formed by the division of that off the parent.

When the number of protoplasts formed in this way is comparatively small, usually four or eight, on release they soon develop a cell wall and grow directly into normal *Chlamydomonas* cells. In their formation there is little that differs from normal cell division, only that they are formed

several together within the original cell. This is then a simple process of reproduction, the cells formed within the parent cell are known as spores, and, since they are motile, as zoospores.

In some species the divisions are at times more numerous, and the protoplasts are smaller, sixteen, thirty-two, or even sixty-four, being formed in one cell. Though these small cells may grow directly into a new individual, their growth is frequently

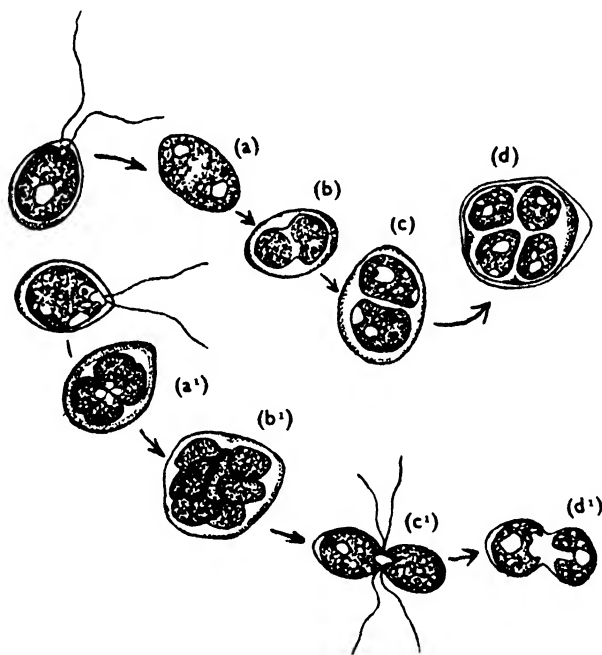


FIG. 138.—*Chlamydomonas*, (a)-(d) stages in cell division in non-motile (*Palmella*) stage, (a')-(d') stages in production of gametes, and conjugation ($\times 600$).

preceded by a fusion ; two of them, which probably originated within different individuals, become entangled by their flagella and then come into contact by their clearer, anterior ends, the protoplasts then coalesce, and finally the two nuclei fuse (Fig. 138). This fusion cell, or zygote, may remain motile for a short time, but soon develops a thick wall, and after a period of rest as a zygospore germinates by the formation of zoospores. The process of union of two exactly similar protoplasts, or gametes, is spoken of as conjugation, and is apparently a simple

sexual fusion. In this simple type of plant all gradations in size and behaviour between normal vegetative cells, zoospores, and gametes, are seen, and it is clear that the naked¹ protoplast of the gamete is organised exactly like the normal vegetative cell, from which it differs only in the absence of a wall, its smaller size, and the fact that its formation is preceded by a number of rapidly succeeding divisions of the nucleus and protoplast.

The Filamentous Alga.

The significance of the conclusion just reached is enhanced when we examine the life cycle of *Ulothrix*. This unbranched, filamentous alga grows in running water and each filament is attached to a stone by a modified basal cell ; all the other cells of the filament are alike and characterised by a band-like chloroplast which forms an incomplete cylinder just within the wall of the cell and has embedded in it a few pyrenoids (Fig. 139a) ; at different times the filaments vary considerably in width. In *Ulothrix* the individual plant is clearly the filament and growth and transverse division of the cells results in increase in length of the individual and not in a multiplication of individuals as in the unicellular types, but after a period of vegetative growth a change becomes apparent progressively down along the filament and which usually affects every cell except the basal cell. The pale and almost uniform green of the vegetative cells changes to a darker and more granular appearance due to the sub-division of the contents into uninucleate masses, which usually range in number, in multiples of two, from two to sixty-four in each cell ; the number tends to be similar along the length of the same filament (Fig. 139). A small aperture then appears in the side wall of the cell, caused apparently by the swelling force developed by the cell contents, which are extruded through the aperture into a delicate, mucilaginous vesicle. The way in which the small, naked protoplasts deform as they are squeezed through the hole suggests that they are almost fluid in consistency ; the vesicle soon disappears and the separate protoplasts, which are very similar to those released from *Chlamydomonas* cells, swim away vigorously. The larger ones formed in small numbers in each cell have four flagella and are macrozoospores, whilst those formed in larger numbers up to thirty-

¹ In some species of *Chlamydomonas* the gametes are walled cells.

two in a cell may have four flagella when they are microzoospores, or two when they may be either microzoospores or gametes. If the motile cell is a zoospore it swims about for a

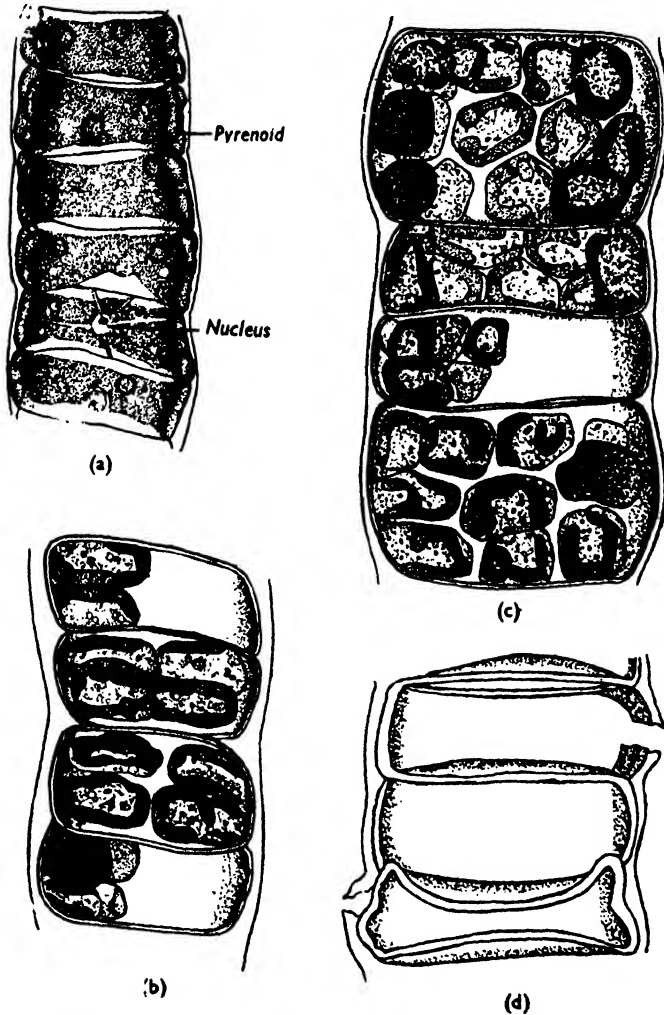


FIG. 139.—*Ulothrix*, (a) cells of vegetative filament, (b) and (c) cells forming zoospores, (d) empty cells after release of zoospores ($\times 640$).

time and then loses its flagella, comes to rest and becomes attached to some object in the water. The protoplast secretes a wall and proceeds to grow into a new filament by repeated

cell divisions. Occasionally the development of the zoospores appears to be arrested before they reach the motile stage and in this case the protoplasts round off and secrete walls without being released ; such aplanospores germinate inside the parent cell but the old wall offers little resistance to the growth of the new filaments.

Gametes are formed in the same way as zoospores, but as many as sixty-four may be formed from one cell ; they are always biflagellate. On release gametes of the same size fuse in pairs, but fusion only takes place between gametes derived from different filaments. The fusion cell, or zygote, swims about for a time with the two pairs of flagella and the two eyespots contributed by the two gametes ; it then comes to rest, loses its flagella (probably by abscission), secretes a thick wall and enters upon a period of rest as a zygospore. On germination the nucleus of the zygospore undergoes successive divisions, the first two of which follow in rapid succession, the protoplast undergoes cleavages into uninucleate parts which round off as aplanospores and which subsequently grow into new vegetative filaments.

Thus we have in *Ulothrix* a beautiful example of a slightly more highly organised plant, which in the reproductive stage forms zoospores or gametes which are strongly reminiscent of the normal motile cell stage of the unicellular alga. Another alga which we examined in the vegetative condition at an earlier stage of our studies, *Spirogyra*, behaves in a different way when it reaches the reproductive stage, when fusion takes place between two gametes which are not liberated into the surrounding water. The filaments, the cells of which it will be remembered were characterised by the striking spiral organisation of the peripheral, band-like chloroplast, grow by increase in size and transverse division of the cells, and the filaments also may multiply when separation occurs between two cells or through the death of a cell. There is formed in this way a thick mat of slippery, unbranched filaments, which float on the surface of still water. In this condition they are often so crowded that the cells of many of the filaments lie in close contact with those of other filaments, and at certain points the walls become more extensible and begin to bulge, so that the filaments are gradually forced apart by the expansion of lateral protrusions. Naturally this

part of the wall also becomes weaker as it expands and ultimately the opposing walls break down at their point of contact, and a continuous canal is formed connecting the cavities of the two cells belonging to the different filaments.

It is a striking characteristic of the cells of a *Spirogyra* filament that they are all usually at about the same stage of their cycle of cell growth ; consequently all the cells of the same filament tend to divide at the same time (usually between 11 p.m. and 2 a.m.), and similarly, when sexual reproduction commences, all the cells that lie in contact with others of a similar filament, reach about the same stage in the reproductive process at the same time.

During the outgrowth of the protuberances and their coalescence to form the conjugation canal, the contents of the two adjacent cells have been undergoing considerable changes ; most of the contents contract, leaving only a thin layer lining the wall, and the spiral chloroplast becomes less distinct. Though all the conjugating cells of the same filament reach the same stage at the same time, in the cells of one of the filaments the contraction of the protoplast takes place earlier, and the outlines of the chloroplast become completely lost, whilst in the other filament the cell contents are later to contract and the spiral nature of the chloroplast still remains discernible. As conjugation proceeds it will also be seen that from the filament of which the cell contents contracted first, the protoplasts are discharged through the conjugation canals into the cells of the adjacent filament. Apparently the liquid contents are at rather a higher pressure in the cells of the former filament and drive the protoplast through the canal. Within the cavities of the cells of the receiving filament, the pairs of protoplasts coalesce and the nuclei fuse. The fusion protoplast, formed in the cell by the union of the two gametes, is still rounded away from the original wall, but soon secretes a new wall and forms an oval, thick-walled zygospore (Fig. 140). Within this zygospore it may still be possible to recognise the spiral organisation of the chloroplast, and upon germination, usually after a prolonged period of rest, the new chloroplast would appear to arise from this existing chloroplast, which has clearly never lost its organised structure since it was contributed by the original passive gamete.

From the behaviour of filaments in conjugation, there is clearly some difference between the two gametes contributing

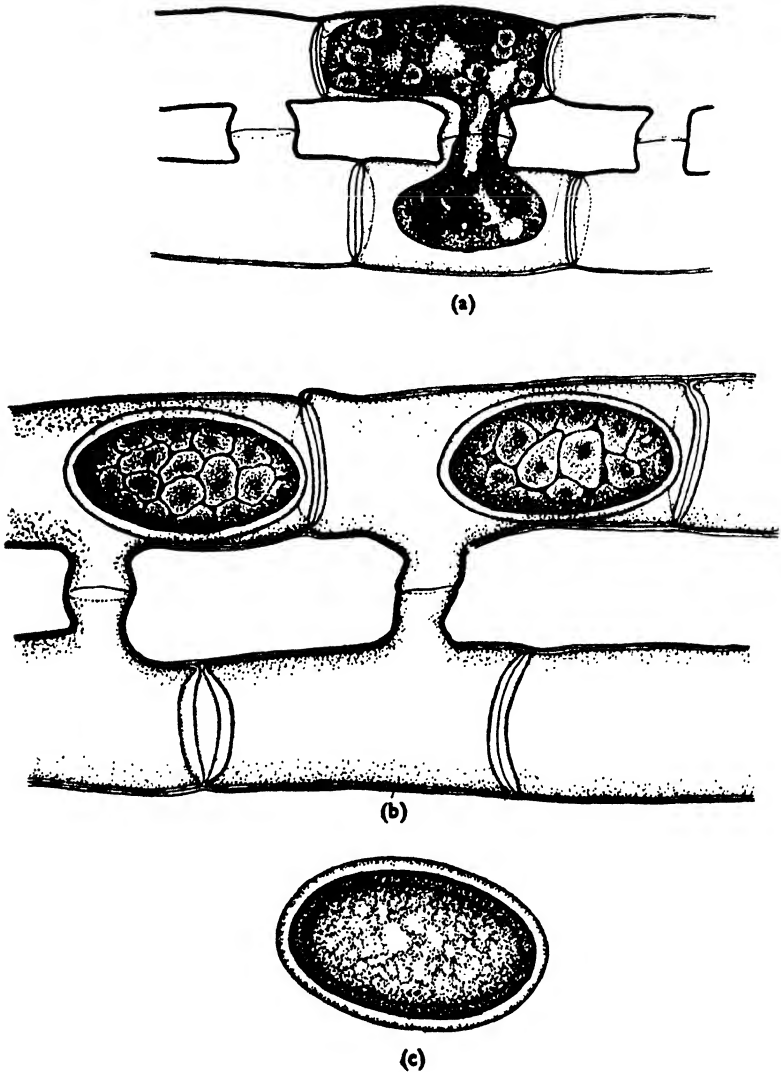


FIG. 140.—*Spirogyra*, (a) cells in process of conjugation ($\times 270$), (adapted from figures by Lloyd), (b) young, and (c) mature, zygospores, starch in the young zygospore, and oil in the mature ($\times 500$).

to the zygote, a difference which extends to all the cells of each individual filament, for as two filaments conjugate side

by side, the zygospores are formed in the cells of one filament only. Furthermore, if two conjugating filaments, A and B, lie side by side over another part of their length with a third filament C, then either A or B may conjugate with C, but not both. Also if of these original filaments A and B, A contained the zygospores, if A conjugates with C, A will again contain the zygospores, whilst if B conjugates with C, then C must be of the other type and will contain the zygospores. In other words the individual filaments of this species of *Spirogyra* might be divided into two categories, one in which the chloroplast undergoes more disorganisation and out of which the contents always migrate, the other in which the zygospores are formed, and where the chloroplast appears to retain and transmit its organisation to the cells of the new filament. Analogies with other types, yet to be considered, make us regard these differences as suggestive of a sex difference between the filaments; the filaments with the more active gametes correspond more closely with the male sex, those with the more passive gametes, and in the cells of which the zygospores are formed, with individuals of the female sex. If this difference of sex is determined during development, then, as both sexes contribute to the formation of the zygote, the sex of the filament must have been determined extremely early in germination, since all the cells of a filament normally behave in the same way. A point which is significant in this connection is that, on germination, the zygospore nucleus undergoes two divisions in rapid succession (in a manner comparable with the divisions of a pollen mother cell), and only one of the resulting nuclei persists as the functional nucleus of the new filament, whilst the remaining three disorganise.

In many species of *Spirogyra* the sex of the filaments is less strongly determined, and in such species adjacent cells of the same filament may conjugate with one another. In contrast to the former dioecious, or ladder-like, type of conjugation, in these species two protuberances grow out in close proximity, on either side of a cross wall, and the coalescence of these to form a conjugation tube enables the protoplast or gamete from one cell to migrate over into a neighbouring cell, in which the zygospore is formed. The result of the monoecious type of conjugation is readily recognised, because the filaments show zygospores only in alternate cells, or in pairs of cells separated by empty cells.

Whilst *Spirogyra* shows a simple type of conjugation, it shows no recurrence of the motile cell stage that we may regard as reminiscent of the motile unicellular alga. It is this latter stage which throws more light upon the significance of the normal sexual process in the higher plants, and we can trace a further stage in its development by the study of the life cycle and sexual organs of a brown seaweed, such as one of the wracks (*Fucus*), so common on rocky shores washed by the tides.

The brown algæ are practically all seaweeds, and amongst these the wracks are comparatively large plants, consisting of "fronds," often 2 or 3 feet long, attached by a holdfast to some firm substratum. The "fronds," though flattened at their free extremities, have no very definite leaf form, and are comparatively simply constructed; each one has a thickened midrib, flanked by a thinner wing, which tends to wear away in older parts. In the centre they are built up of numbers of branched filaments which run mainly along the length of the frond and are embedded in a mucilaginous matrix. The branches of the filaments continually bend outwards and graduate into a smaller celled peripheral region, the cells of which contain relatively more protoplasm and more inclusions, such as the pigment-containing granules, the chromatophores. These smaller cells continue to divide, and so growth continues all over the surface of the fronds, whilst the inner cells are continually elongating and contributing to the mass of interlacing filaments which compose the central part.

Though this plant is differentiated into a branched holdfast at the base, a cylindrical stipe from which the wings of the frond have worn away whilst the central region continued to thicken, and the repeatedly forked series of flattened fronds, it is still not differentiated structurally as a vascular plant, and the vegetative body is termed the thallus. The forked fronds are evidence that the apical growth of the free ends of the thallus is associated with a frequent, equal division, or dichotomy, of the growing region.

One very striking difference between the thallus of the brown seaweeds and the vegetative body of the higher plant, is that in the seaweeds there are no air-filled intercellular spaces. This is apparently the result of a curious metabolism, in which enormous quantities of mucilaginous substances

are secreted and completely fill the intercellular spaces. As a result, though the walls of the constituent filaments lie well dispersed from one another in the central region of the thallus, all the intervening space is filled with mucilage. At the surface of the thallus the growing cells are closely packed together, and the walls are thin, so that the internal mucilage does not readily diffuse away through this close-packed external surface, though the wracks, on a damp day, will always be found very slimy to the touch.

In the bladder wrack, *Fucus vesiculosus* L., gas collects in certain localised positions in the fronds, frequently just beneath a fork. The bubbles thus formed expand and displace the central filaments until these form a more compact layer limiting the cavity thus created. Many bladders are developed in the thallus of this species and presumably help the fronds to float or stand erect when submerged, but other species of wrack, such as the serrated wrack, *Fucus serratus* L., grow equally well without any bladder formation; the latter species normally grows at a lower level in the tidal zone than *Fucus vesiculosus* L.

Many other points of interest will be observed when the plants are examined, but we are concerned mainly with the process of reproduction in the group. In the wracks the sexual organs are borne in rounded pits, sunken into the tissues of the fronds. These are crowded together towards the distal lobes of the forked thallus, which are considerably swollen; similar, though sterile cavities, which contain hairs only, are also scattered over the surface of the plant. The flask-like cavities, or conceptacles, open to the exterior by a mouth or ostiole, whilst the sexual organs are borne amongst sterile hairs which are active in the secretion of mucilage.

In all the wracks there are two types of sexual organ, the male and female, and though in some species these may be borne in the same conceptacle, in the two species considered here they are borne on different plants. From the internal wall of the female conceptacle long, almost unbranched hairs grow out, and amongst these are numerous female reproductive organs, the oogonia. The oogonium is a single large, oval cell arising from a dome-shaped cell of the conceptacle wall (Fig. 142a); its contents divide up into eight uninucleate parts, the eggs or ova. In the male conceptacle the

cavity contains numerous branched hairs, on each of which are borne several oval bodies, the male organs or antheridia, each of these, though much smaller than an oogonium, gives rise to sixty-

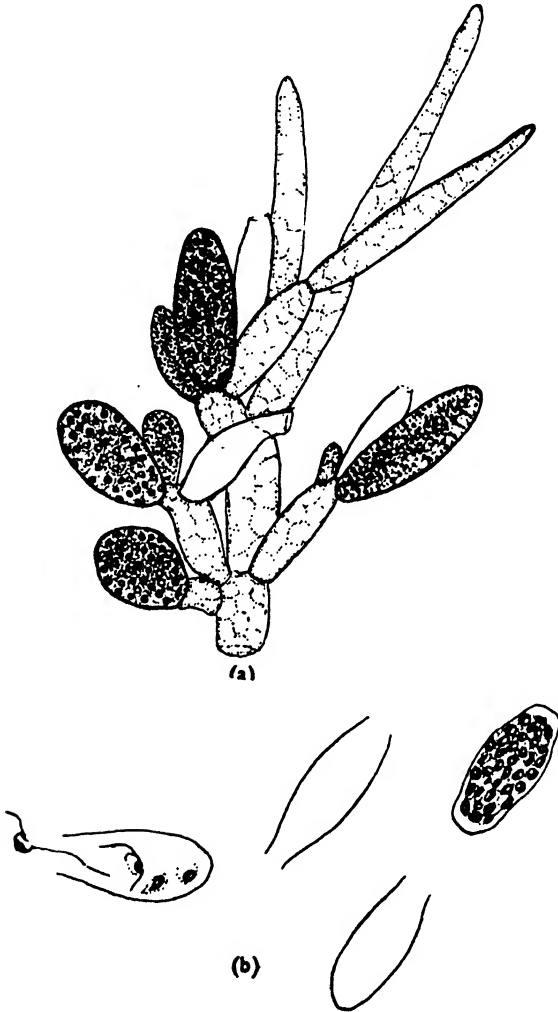


FIG. 141.—*Fucus serratus*, (a) branched hair from male conceptacle bearing antheridia and empty sheaths from which sperm packets have escaped, (b) packets of sperms released from antheridia. The discharge of the sperms leaves the empty inner layer of wall ($\times 525$).

four uninucleate portions, the sperms (Fig. 141). When the algæ are exposed between tides, the outer tissues tend to dry and contract, and pressure is exerted on the mucilage in mature

conceptacles, this oozes out as small droplets through the ostiole. At this stage the plants of the two sexes may easily be recognised, as the swollen ends of the male fronds are covered with bright orange droplets, whilst the droplets on female plants are dark green, due to the contents of the mature antheridia and oogonia respectively, which are exuded with the mucilage. In each case the outer wall layer of the oogonium or antheridium bursts and remains attached respectively to the floor or hair in the conceptacle, and the contents are expelled, still enclosed in an envelope composed of the inner layers or layer of the wall (Fig. 141).

Under natural conditions, when the tide rises again, this mucilage becomes dispersed in the water; the changes which occur may be followed, however, if some of the mucilage from the two kinds of plants is mounted in a drop of well-aerated sea-water, even if the plants have been kept in a tin for one or two days after collection. Under the microscope the packets of eight ova are very conspicuous with their dense, dark green contents (Fig. 142*a*). If these are watched, with the light well controlled, so that the very transparent, swollen wall can be seen, it will be noted that the outer layer of the wall, still enclosing the eggs, bursts first at the distal end, and, as the contents, still enclosed in an inner mucilaginous sheath, emerge through the opening, the outer burst layer is almost everted (Fig. 142*b*). The inner layer soon disappears, and the ova are left free in the water. Whilst enclosed in the oogonium the ova appear angular owing to close contact with one another, but as soon as they are released each naked protoplast rounds itself off into a sphere, large enough to be visible to the unaided eye (Fig. 142*c*). The wall of the antheridial packet is only single-layered, and soon bursts in water liberating the contents (Fig. 141*b*). These are very small, but under the microscope the sperms are recognisable as minute pear-shaped bodies, each of which contains a bright orange dot, formed originally from the chloroplast. As soon as they are liberated they begin to swim about vigorously by means of two unequal flagella, which are attached to the side of the cell (Fig. 142*c*.) The eggs are evidently centres of attraction for the sperms, as they are seen to collect in large numbers around the eggs, with the surface of which each sperm makes contact by means

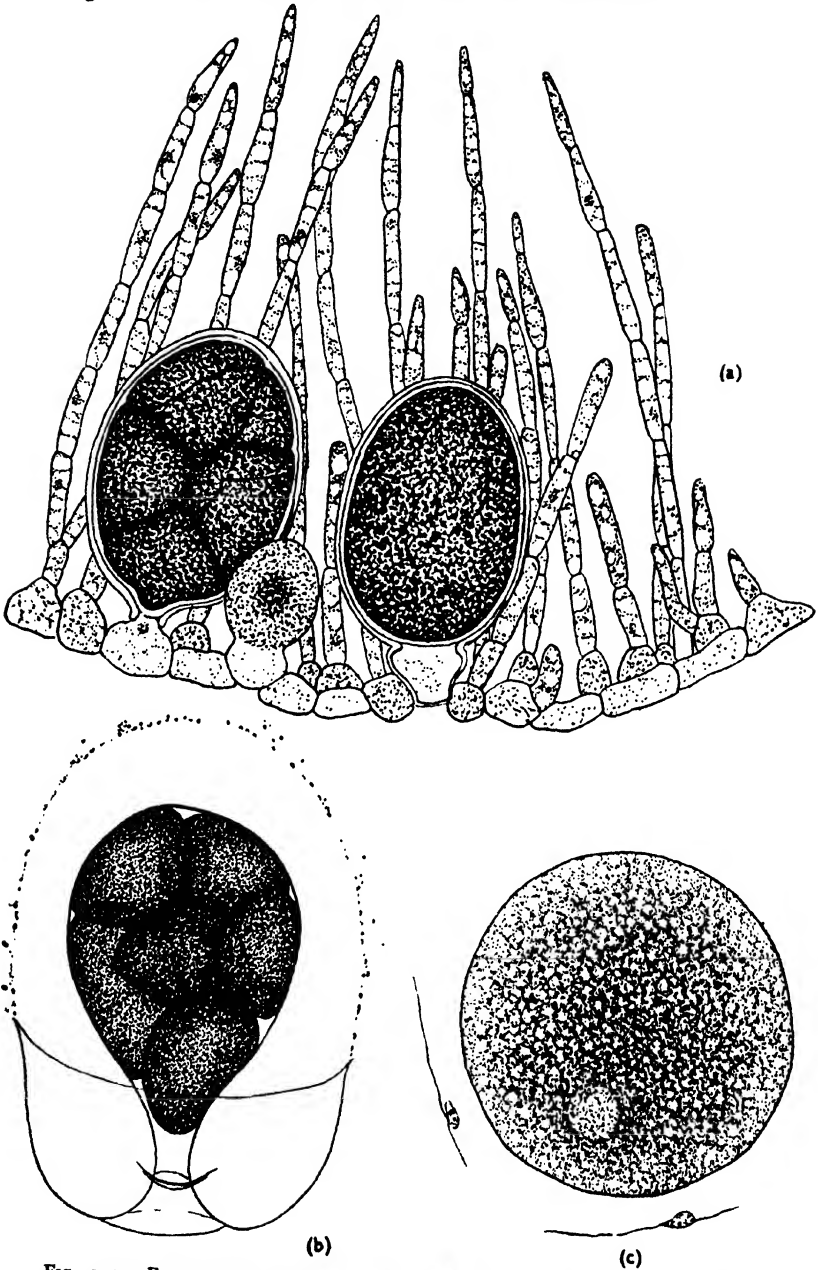


FIG. 142.—*Fucus serratus*, (a) Oogonia and hairs from the oogonial conceptacle ($\times 90$), (b) packet of ova released from the conceptacle, the middle wall layer burst, ova still retained in inner wall layer ($\times 100$), (c) ovum free in water with two sperms ($\times 480$).

of its forwardly directed, shorter flagellum. The free flagella of the sperms lash so vigorously that the large eggs are seen to be spinning round in the water. This continues for some minutes, and then suddenly the sperms fall away from the egg, and this is found to mean that one has penetrated the egg; immediately this happens the egg secretes a pellicle around the protoplast. The small sperm penetrates through the protoplasm and eventually its nucleus fuses with that of the egg and fertilisation is accomplished. The fertilised ovum surrounds itself with a cell wall and the resulting oospore soon attaches itself to the rock, and begins to grow into a new plant, which will again bear either antheridia or oogonia.

Mosses.

The types examined up to now have been plants which grow in water and in which, with the exception of *Spirogyra*, fertilisation takes place in the water outside the parent plant, the two gametes being brought together by the swimming motion of one, or both, of the gametes. The mosses may be taken as our first example of plants which grow on land. One of the most convenient to examine is the moss *Mnium hornum* L., which is extremely common in woods, and especially along the banks of streams.

The leafy stems are about an inch in height, with crowded leaves, which are roughly about three times as long as wide. Early in May it is found that, in certain patches, the bud-like apices of the plant have opened out, and are now occupied by a cluster of brownish structures, surrounded by a rosette of leaves (Fig. 143). These are the male plants, and if some of the brown central part is teased out in a drop of water, it is found to consist of a number of characteristic, brown hairs, amongst which are the antheridia. These are dark green, elongate bodies, each borne on a multicellular stalk. In water the inner layers of the outer wall of the large cap cell of the antheridium swell and the resulting pressure causes the inner wall to be ruptured and the contents to be discharged into the antheridial cavity; at the same time other wall layers and the contents of the antheridium as a whole are also swelling and finally the remaining swollen wall of the cap cell is burst and the contents emerge as a long, grey coil built up of very large

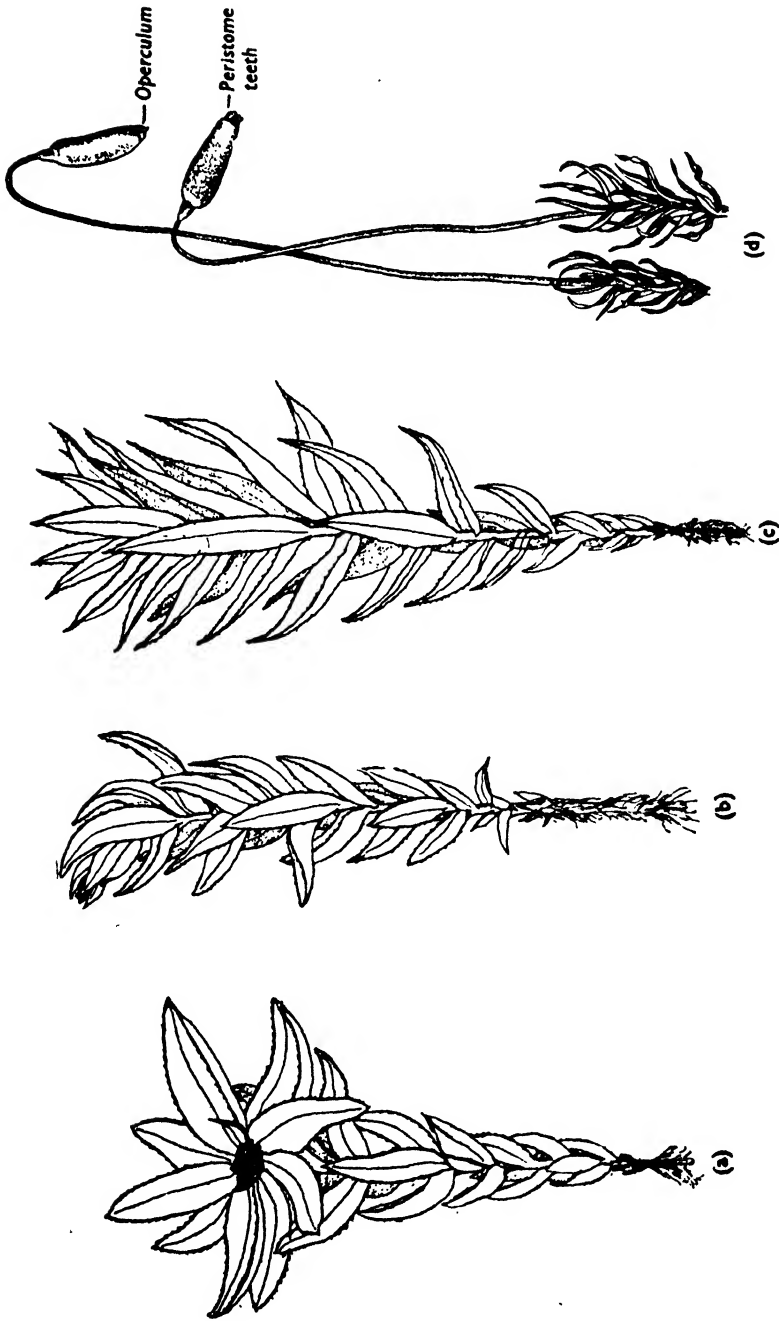


FIG. 143.—*Mnium hornum*, (a) male, (b) vegetative, (c) female, shoots ($\times 3$), (d) shoots with capsules ($\times 2$).

numbers of minute cubical cells. The wall of the antheridium may then be seen to consist of a layer of sterile cells containing chloroplasts (Fig. 144). If the antheridium is quite mature, it may be seen that movement is taking place in each extruded cell in which a small elongate body is rotating round and round. After a time the walls of these cells swell and weaken, and from each cell there escapes a single sperm, which has an elongated body, composed almost entirely of a nucleus, to one end of which is attached a round protoplasmic vesicle, and to the other a pair of delicate flagella, by means of which the sperm swims about actively. In *Mnium*, the period over which the antheridia are in the right condition to show the motility of the sperms appears to be very short, but this stage may usually be obtained in another moss, *Polytrichum*, in May.

In other patches of *Mnium* there are none of the cup-like heads characteristic of the male plants, and all the plants are terminated by pointed leafy buds. The sterile shoots, in such a patch, have buds in which the apex is occupied by progressively smaller and younger leaves, whilst other shoots are terminated by a group of relatively long and darker green leaves (Fig. 143). If the buds of this latter type are dissected, the centre is found to be occupied by a group of the female organs, or archegonia (Fig. 145). Each archegonium has a massive stalk, above which the structure expands slightly to form the venter, which again narrows distally into the long neck. Within the venter lies the ovum, and above it the ventral canal cell, whilst in the cavity of the neck lie a series of canal cells which subsequently break down into mucilage. All these cells can be clearly seen in the young archegonium if the preparation is mounted in Eau de Javelle. In contrast to the alga, the ovum of a moss is not discharged; at maturity the neck canal cells disorganise and the apex of the neck bursts open, sperms are attracted by substances released from the neck and the ovum is fertilised *in situ*. Though this later part of the journey of the sperm to the egg is a relatively simple matter, the way in which the minute sperm is transferred from a male to a female plant is more difficult to explain. It is not surprising that archegonia are seldom fertilised unless male plants occur near-by, or preferably are intermixed in the same patch. In 1942 Walton showed that if mature antheridia are mounted in water, without a cover-glass, they

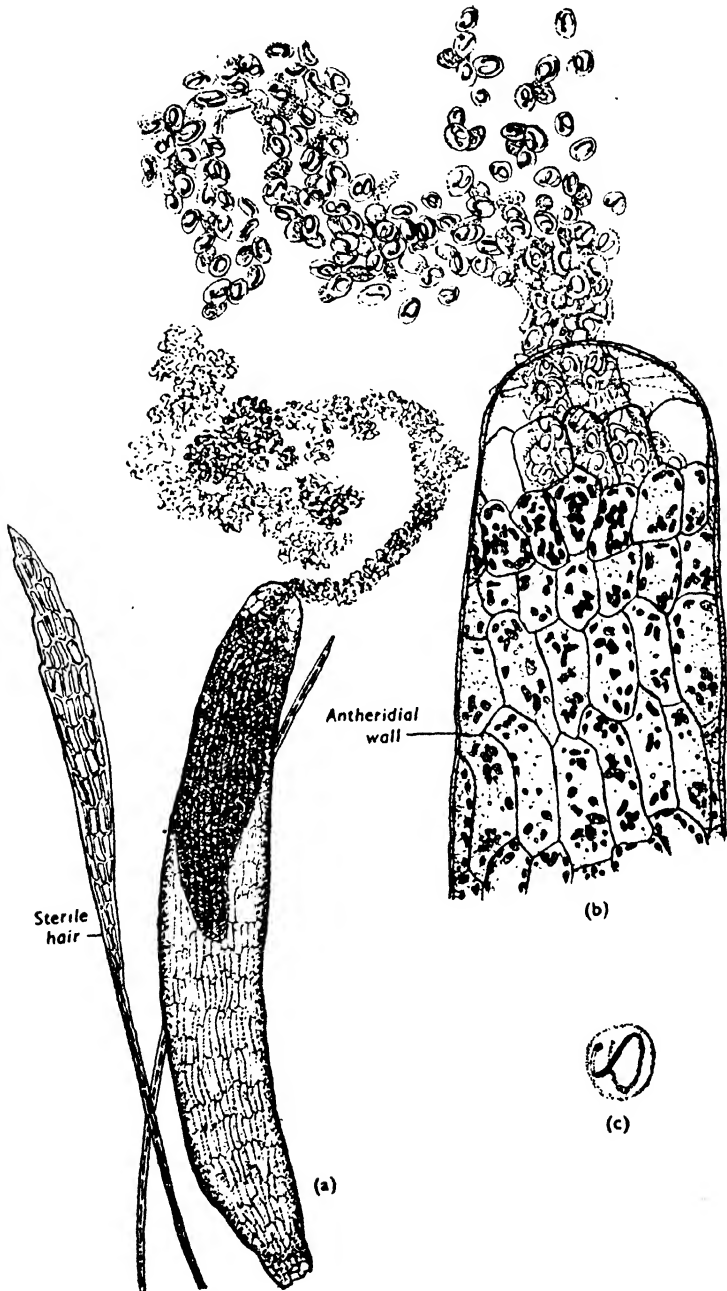


FIG. 144.—*Polytrichum*, (a) antheridia and hairs from male plant ($\times 60$), (b) antheridium discharging sperm mother cells ($\times 275$), (c) (sperm in mother cell ($\times 850$)).

soon dehisce and the contents move to the water-air surface where the sperm mother cells become uniformly distributed in the surface film. The sperms are then released from their enclosing cells. The same process occurs when a drop of rain collects in the leafy cup at the top of the antheridial plant and sperms may conceivably be transferred from the surface film to the archegoniate shoots by the splashing of rain drops, or

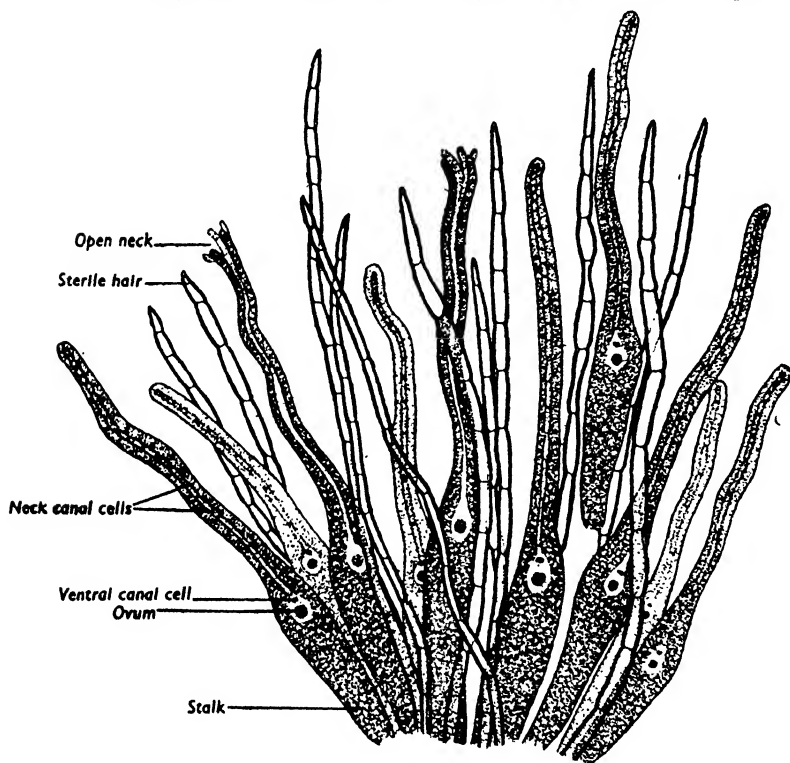


FIG. 145.—*Mnium hornum*, hairs and archegonia mounted in Eau de Javelle ($\times 80$). through the agency of small insects which are usually to be found in the moss shoots, where they probably feed on the mucilage from the reproductive organs. When the drop of water in the cup subsequently dries, the discharged antheridial contents form a glistening film over the surface of the perichaetial leaves.

It will be seen that in this type, as in *Fucus*, the two gametes, the ovum and the sperm, differ greatly in size, and that motility has been retained by the sperm but lost by the ovum. The moss type differs, however, from the alga, in that the gametes

are enclosed in the reproductive organs by a wall composed of cells, the wall of the antheridium, and of the venter and neck of the archegonium. The fertilisation of the ovum *in situ*, and the germination of the oospore in this position is probably significant in relation to the subsequent behaviour of the structure arising from the oospore, for it has of necessity to draw most, if not all, of its food supplies from the leafy female shoot upon which it is growing, and the structure into which it develops is entirely different from the leafy plants of either sex. The oospore

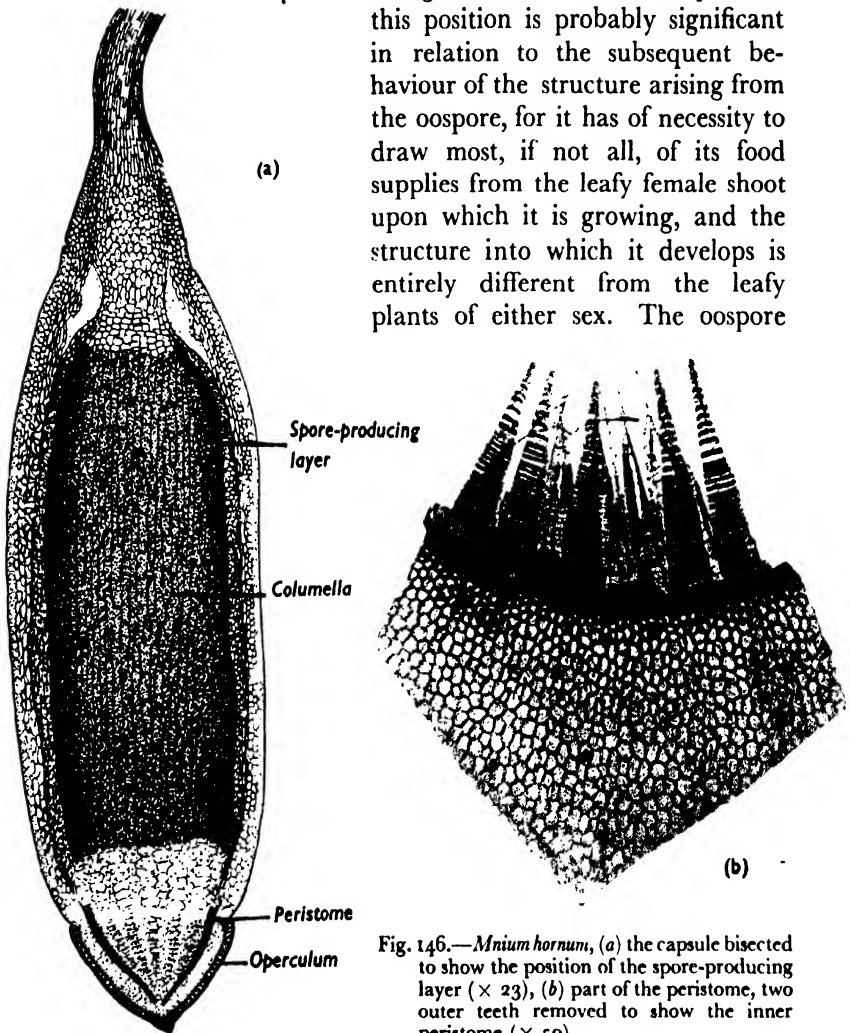


Fig. 146.—*Mnium hornum*, (a) the capsule bisected to show the position of the spore-producing layer ($\times 23$), (b) part of the peristome, two outer teeth removed to show the inner peristome ($\times 50$).

forms a downward, conical prolongation, known as the foot, which buries itself in the tissues of the leafy female shoot, and through this the food must be absorbed, which is used in the production of a stalk-like region, the seta, about 2 inches in length in *Mnium hornum*, and the swollen spore capsule

(Fig. 143*d*). The immature capsule in April is green and the part which adjoins the seta and also a distal conical part are entirely sterile. Along the wider part, between a sterile wall region and a central sterile columella, a narrow cylinder of cells gives rise to spores (after the division of the spore mother cells into tetrads of spores) (Fig. 146*a*), and the spores are finally liberated by the separation of a lid or operculum as the capsule dries. When the lid falls, the opening of the capsule is still partially closed by a double ring of strips of thickened cell walls, known as peristome teeth, which only allow the spores to be shaken out and blown away under dry conditions (Fig. 146*b*). If the ripe capsule, with the lid removed, is fixed upright on a microscope slide by pushing its base into a little plasticine, the peristome can be studied by reflected light, when it will be seen that the inner teeth hardly alter their position, but the outer teeth curl inwards when the air is moist (brought about by breathing on the capsule under observation) and catch between the teeth of the inner peristome. It is probably the jerky movements of these teeth, as they free themselves again on drying, that are largely responsible for spore dispersal in pendent moss capsules such as *Mnium*. The spores germinate freely on moist soil, and, after a preliminary filamentous stage, give rise to the leafy plants bearing male or female reproductive organs.

The liverworts have a life cycle essentially similar to that of mosses, and it will be seen that in either of these two groups, a new feature is introduced by the regular alternation of two distinct kinds of plant, each starting from a single cell stage. The spores grow into the plants, which finally produce the gametes, the sperms and the ova. These thalloid or leafy plants are consequently known as the gamete-bearing plants, or gametophytes. After fertilisation development again starts from a single cell, the oospore, and this grows into the spore-bearing plant, or sporophyte, which in these types is largely dependent upon the gametophyte, upon which it develops. The regular alternation of these two generations is something new in the life cycle, and it seems to be coupled with the retention of the ovum, and resulting oospore, within the archegonium. In the algæ, the rapid multiplication of such types as *Ulothrix* was brought about by zoospore formation; in mosses and liverworts the plants are multiplied mainly by the wind-dispersed spores, a method more suited to a land plant.

CHAPTER XXXVI.

LIFE CYCLES THROUGHOUT THE PLANT KINGDOM.

II. VASCULAR PLANTS. FERNS, GYMNOSPERMS, AND ANGIOSPERMS.

Ferns.

In the life cycle of the fern it is possible to recognise the same alternation of spore-bearing and gamete-bearing plants as in the mosses. The male fern (*Dryopteris Filix-mas* Rich.) is a large plant growing on land, and with the same morphological members, leaf, stem, and root, as in the flowering plants. The stem is short and thickly covered with the bases of old leaves, from amongst which grow out a number of branched roots. Each year the plant produces a crop of about twelve to twenty large, compound pinnate fronds, of which the growing tips are coiled inwards. The various parts of the vegetative plant are supplied with veins, in which xylem and phloem are present, whilst even in the largest mosses the central strand, which may be present in the stem or in the leaves, is composed of elongated cells which may serve for conduction, but which have not the features characteristic of xylem and phloem as found in the flowering plants and ferns.

Young fern plants have fewer and smaller leaves, and these are sterile, but as the plant grows year after year, the fronds produced are progressively larger and more numerous, and also develop clusters of spore cases or sporangia on the lower side. These clusters, sori, lie over forkings of the veins on the ultimate pinnæ of the fronds and, as about seven or eight may be developed on each pinna (Fig. 147), the total number on a frond may be very large. In early summer the sorus is recognised by the presence of a pale green, kidney-shaped scale (the indusium), which covers the developing sporangia, but later in the season the indusium shrivels, and the maturing sporangia turn dark brown, giving

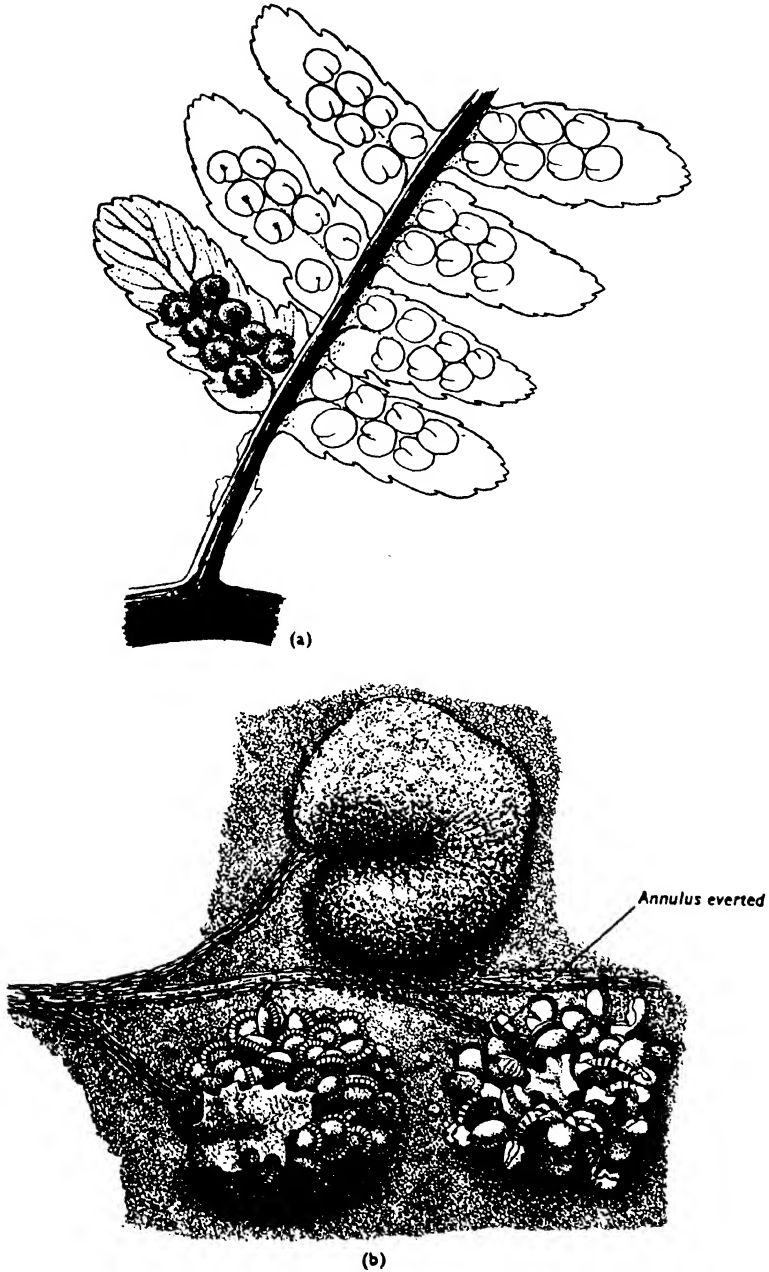


FIG. 147.—*Dryopteris*, part of a leaf with sori on the lower surface (a) ($\times 4$), (b) in lower sori some sporangia dehiscent ($\times 40$).

the sorus the appearance of a small powdery patch. In the male fern a whole succession of sporangia develop in every sorus, so that when the first ones are mature, and are shedding their spores, young ones are still in process of development.

The sporangium has a slender stalk, surmounted by a flat, oval spore case, the flatter sides bulging to give the narrow view a biconvex outline (Fig. 147*b*). The sporangium wall is one cell thick and lined by a layer of denser tapetal cells, which surround a central group of still denser cells, known as the archesporium. Division continues until there are sixteen of the central cells, which, on account of their subsequent behaviour, are known as spore mother cells. At this stage the sporangium enlarges and the sixteen cells round off from one another and lie free, surrounded by liquid, in a cavity which is enlarged by the progressive breakdown of the tapetum. The spore mother cells then undergo two divisions in rapid succession to form the spores, which are at first grouped tetrahedrally in fours. During this process of spore formation, changes also occur in the wall of the sporangium; the annulus, a single line of cells along the narrow margin of the sporangium, extending from the stalk on one side over the top and half-way down the opposite side, become markedly thickened on their inner tangential and radial walls, whilst the outer wall remains thin. On the rest of the narrow margin and on the wider faces of the sporangium, the superficial cells are all thin-walled. As the sporangium dries, the thin outer walls of the water-filled cells of the annulus are pulled inwards until the strain causes the wall of the sporangium to be torn open between two of the thin-walled cells. The strip of annulus cells continues slowly to straighten out and to become everted as the volume of the liquid contents is still further reduced (Fig. 147*b*), until finally the tension on the liquid contents becomes so great that suddenly a bubble of water vapour is formed in each cell and the annulus snaps back to its original position, violently scattering the spores in the process. This dehiscence of the sporangia may be watched under the microscope if a fern frond is selected with dark coloured sori (material pickled in alcohol can be used for the purpose) and a piece is mounted dry and examined by reflected light. The dehisced sporangia are readily recognised, they have a lighter appearance as they contain air.

The spores are very small, and their mechanism of dispersal ensures that they are scattered under dry conditions, when a slight breeze will help to carry them to a distance from the parent plant. Spores which fall into moist positions soon germinate and grow into a flat, heart-shaped, green plant, known as a prothallium. The whole prothallium, even when well grown, scarcely exceeds a centimetre in either direction, and is simple in construction, being composed of parenchymatous tissue without any trace of a vein system; it is fixed to the soil by numerous unicellular hairs (rhizoids) which grow out on the lower side (Fig. 148).

It is best to collect the prothallia some weeks before they are required and to keep them on slightly moist soil in a Petri dish, adding no water from above. Soon after germination the prothallium commences to form antheridia on the under surface. An antheridium projects as a small rounded structure from the cell from which it is formed; it consists of a wall of two ring-shaped cells and a cap cell, surrounding a central group of about thirty-two sperm mother cells, in each of which a single sperm is formed. To see these, a prothallium, and preferably a small one, is taken from the Petri dish and mounted in a drop of water with the lower side uppermost. The antheridia are seen amongst the rhizoids, and some will be ripe in prothallia taken from dry soil. In the ripe antheridium the cap cell will be seen to burst, and the mother cells to swell and emerge. Soon the wall of the mother cell dissolves and the sperm, a spirally coiled body bearing very numerous flagella, is liberated, and swims very actively with a corkscrew motion.

Just behind the sunken apex of a larger prothallium, examined in the same way, may be seen the archegonia. These have the same general construction as in the mosses, but the venter containing the ovum is slightly sunken in the tissues of the prothallium, and the neck is very much shorter and curved away from the apex of the prothallium (Fig. 148). In young archegonia the tip of the neck is closed, but as they mature the disorganised contents of the neck canal cells swell on access of water, and the neck is forced widely open. If prothallia, with both types of reproductive organ, are mounted together at the right stage, it is possible to see sperms swim towards the archegonia, where they appear to be attracted to

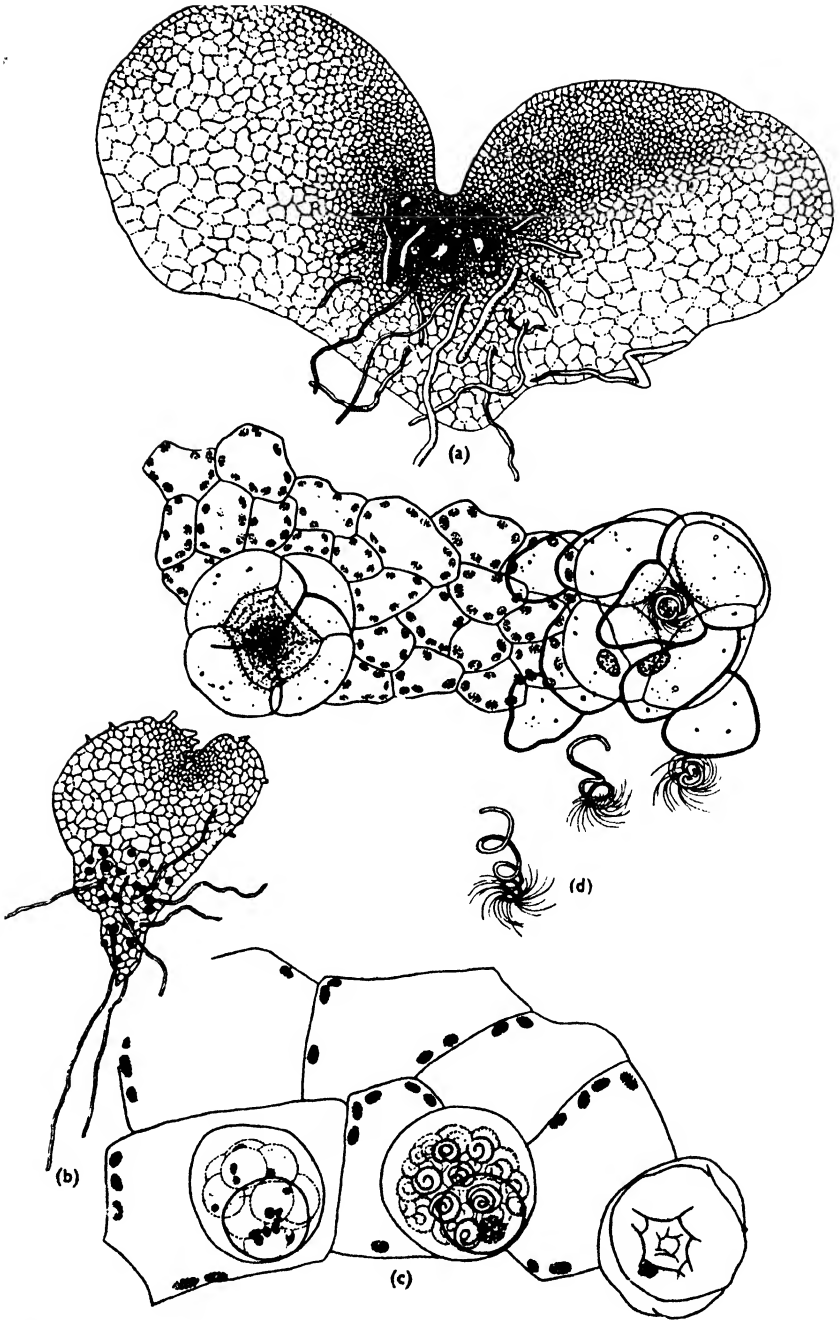


FIG. 148.—*Dryopteris*, lower surface of prothallium, (a) with archegonia, (b) with antheridia ($\times 20$), (c) antheridia, young, mature and discharged ($\times 370$), (d) closed and open archegonial necks ($\times 550$), a sperm has entered the open neck and others, shown rather larger, are swimming near it.

the exuded mucilage and to enter the open neck. At first the bodies of the sperms are closely coiled, and they move very rapidly. The attraction to the archegonia is evidently strong, and frequently the number of sperms trying to enter the neck of the same archegonium may be so large that the neck becomes completely blocked by them. They become less coiled as they enter the archegonium, and their elongated bodies appear as a brush protruding from the neck. It is often possible to focus down the neck canal, when it is seen that some sperms have reached the venter, where they continue to spin round and round for some time. Eventually movement ceases, which probably means that a sperm has penetrated the ovum.

The fertilised egg, oospore, germinates *in situ*, deriving its food from the prothallium. The embryo plant has as its most conspicuous parts a first leaf and a root, but in addition a rounded outgrowth, the foot, grows into the prothallial tissues as an absorbing organ, and a small stem apex is also differentiated between the foot and the first leaf. The first leaf is of simple form, and soon pushes up between the two lobes of the prothallium. The small plant soon becomes independent of the prothallium, and as more green leaves are formed upon it, it builds up, year after year, a larger plant, until eventually it reaches the condition of the adult spore-bearing plant with which we commenced our description of the fern.

In the life cycle of the fern we have the same regular alternation of spore-bearing and gamete-bearing plants as in the mosses, but the relative balance of the two generations has been reversed, so that the large leafy sporophyte in the fern is comparable with the small sporophyte in the moss, where it had almost the appearance of a fruit body growing on the leafy gametophyte, and conversely the leafy moss plant is equivalent to the insignificant prothallium of the fern. The presence of a vascular system, and the method of multiplication by air-carried spores, are both features which make it possible for ferns to multiply as land plants, whilst the other generation, in which water is still essential to enable the sperms to swim to the ovum, is reduced to minor proportions.

In the large-leaved ferns the spores are all alike, and on germination the prothallia produce, first antheridia, and then archegonia, though small, ill-nourished prothallia may not develop beyond the stage of antheridia formation. In the

horse-tail (*Equisetum*), which is grouped with the ferns, all the spores look alike, but on germination prothallia tend to be distinguished into small ones, which bear antheridia, and larger ones bearing archegonia. This tendency to separate the two sexes is developed still farther in yet another group of related plants, the club mosses, of which *Selaginella* may be taken as an example.

One species of *Selaginella* grows in Britain, but another

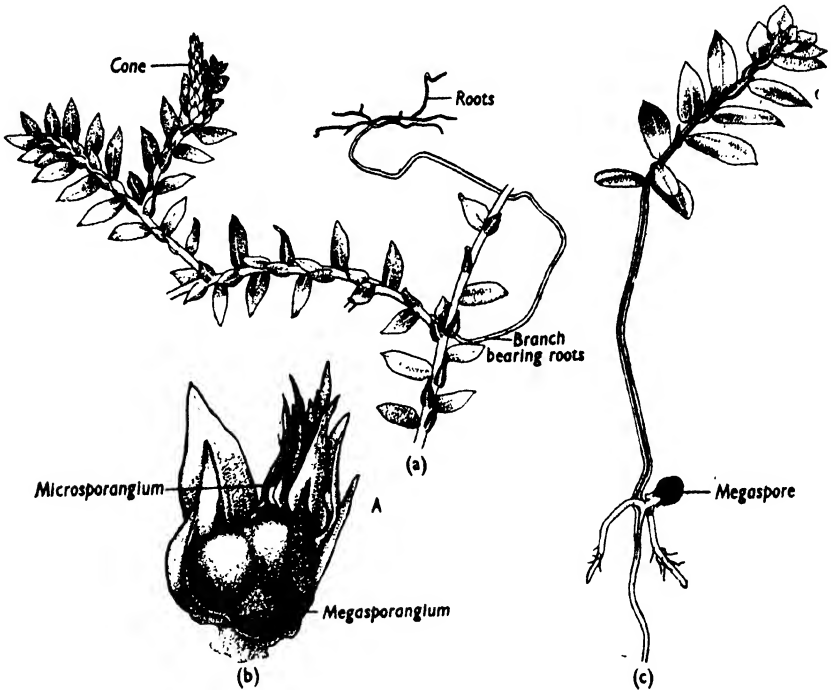


FIG. 149.—*Selaginella Kraussiana*, (a) part of the plant, seen from above, showing dorsiventral habit, leafless branch bearing roots, and a strobilus ($\times 3$), (b) megasporangium and microsporangia on a strobilus ($\times 16$), (c) young plant arising from the prothallium ($\times 6$).

species, *Selaginella Kraussiana* A. Br., native to S. Africa, is so common in green-houses that it is usually more accessible than the wild species. This species of *Selaginella* has a creeping branched stem, thickly covered with very small leaves. Curious leafless branches also grow down from the lower side of the stem and bear roots when they come in contact with the soil. The vegetative stem bears two rows of larger,

ventral leaves, and two of smaller, dorsal leaves. The sporangia are borne in the axils of leaves in small, erect, radially organised cones, on which the leaves are all similar, and more crowded than on the vegetative parts (Fig. 149). The cones contain sporangia of two kinds, those in the axils of the lowermost two or three leaves are large, and much distorted in shape by the four large megasporos within. The sporangia higher on the cone are much smaller, each sporangium being of about the same size as a single spore in the lower sporangia, and in these are contained a large number of very small spores, distinguished as microspores. The two types of spore give rise to two kinds of prothallium. The large megasporos have an appreciable amount of food in them and begin to grow whilst still within the sporangium. The original single nucleus divides repeatedly, and walls subsequently form between the nuclei so that the spore contents become divided up into a small-celled tissue, the formation of which starts at that side of the spore on which a tri-radiate marking is present, indicating the position where the four megasporos were in contact with each other in the tetrad. Finally the whole spore cavity becomes partitioned up, the spore coat ruptures along the tri-radiate ridges, and the small, almost colourless lobes of the prothallium protrude through the clefts. On this part of the prothallium are formed the archegonia, which are similar to those of other ferns, but are sunken in the tissues so that only the tip of the neck is exposed; the first archegonia begin to appear shortly before the spores are shed.

The microspores grow into extremely minute prothallia. The first division in the germinating spore, the only one to take place before the spore leaves the sporangium, cuts off a minute cell, which takes no further part in the story of development, and which is all that remains to represent the vegetative part of the prothallium. The rest of the spore divides up into about a dozen cells which represent a single antheridium with eight wall cells surrounding four central cells, in each of which a sperm is developed. After a time the wall cells disintegrate, the rounded sperm mother cells lie free, and as the wall breaks down in water, the sperms are liberated. Each sperm consists of an elongated body, composed mainly of the nucleus, with two protoplasmic flagella attached at the forward end. In a thin film of water these

sperms swim freely, and if they reach the neighbourhood of a female prothallium they are attracted to the archegonia. After fertilisation, the oospore grows into a characteristic embryo, the main portion of which is pushed down into the food stored in the prothallium by the growth of a few-celled suspensor, developed towards the neck of the archegonium. The embryo consists of a root, stem apex, a pair of leaves, and a foot, through which food is absorbed from the prothallium.

In the case of *Selaginella*, the heavier megaspores fall to the ground, whilst the more numerous and lighter microspores are dispersed by the wind, and for fertilisation to be effective, it is probably essential that the microspore should germinate in close proximity to the female prothallium. Since the male prothallium is little more than an antheridium forming sperms, in this case the greater part of the journey of the sperms to the archegonium is traversed in the form of a wind-carried spore, and the dependence on water is reduced to a minimum, though the sperm has still to swim the last small part of the journey.

In the male fern the prothallium soon commenced to produce antheridia, whilst archegonia were only formed on better grown prothallia when they had been assimilating in the light for some time, and therefore more food was available. In *Selaginella*, on the other hand, no assimilation is carried out by the male prothallium, and very little by the female, so that the prothallial development is almost entirely dependent upon the food, derived from the leafy sporophyte, which was stored in the spore itself; the microspore, with its small amount of food, gives rise to the male prothallium with the antheridium, and the megaspore, with larger reserves of food, forms the female prothallium. Since the food supplies present in the female prothallium are almost entirely those passed into the megaspore from the previous sporophyte, when the embryo sporophyte commences to grow at the expense of the prothallium, it is being nourished indirectly by the previous sporophyte. In some species of *Selaginella* only one megaspore, instead of the usual four, may develop in the megasporangium, and also in some types the megaspore is not shed from the sporangium, and fertilisation is effected by sperms from microspores, which have been caught between the cone scales near

to the megasporangium. If the megaspore regularly remained and germinated in the sporangium on the parent plant, and the young stages of the new sporophyte were also passed in the female prothallium, in the megasporangium on the previous parent sporophyte, we should have reached the type of life cycle that is shown by the seed-bearing plants; but before attempting to draw a detailed comparison between the ferns and the angiosperms, the gymnosperms should be examined.

Gymnosperms.

In one group of the gymnosperms, the cycads, the plants have a fern-like habit with a relatively short stem bearing a crown of large, pinnate fronds. The sporangia-bearing leaves, or sporophylls, are smaller than the vegetative, and are grouped into cones. In *Cycas revoluta* Thunb. the megasporophylls are still pinnate, and in the female cone the megasporangia are borne along the margins. Each megasporangium is large and is enclosed in a cup-like upgrowth, which one may compare either with the indusium of the fern or the integument of an ovule. The microsporophylls are more reduced and are represented by closely-fitting cone scales, the lower surfaces of which are covered with crowded microsporangia. On dehiscence the microspores are dispersed by wind and some are caught in the micropyle of the ovule, where they germinate in close proximity to the archegonia of the female gametophyte which has developed in the ovule. In this position the microspore produces a pollen tube, which grows down towards the archegonia, but before the tube quite reaches them, the tip ruptures and two sperms are liberated. The sperms are relatively large ovoid bodies, which bear very numerous cilia, arranged in the form of a spiral band running round the sperm from the more pointed end, and by means of these the sperms swim the last part of the journey to the ovum in the archegonium. In the cycads and in the maiden-hair tree of Japan (*Ginkgo biloba* L.), though the microspores or, as we may now call them, the pollen grains, are carried by wind to the ovules, and then the male cells are carried nearer to the ovum by the growth of a pollen tube, when these male cells are finally liberated, we find that they

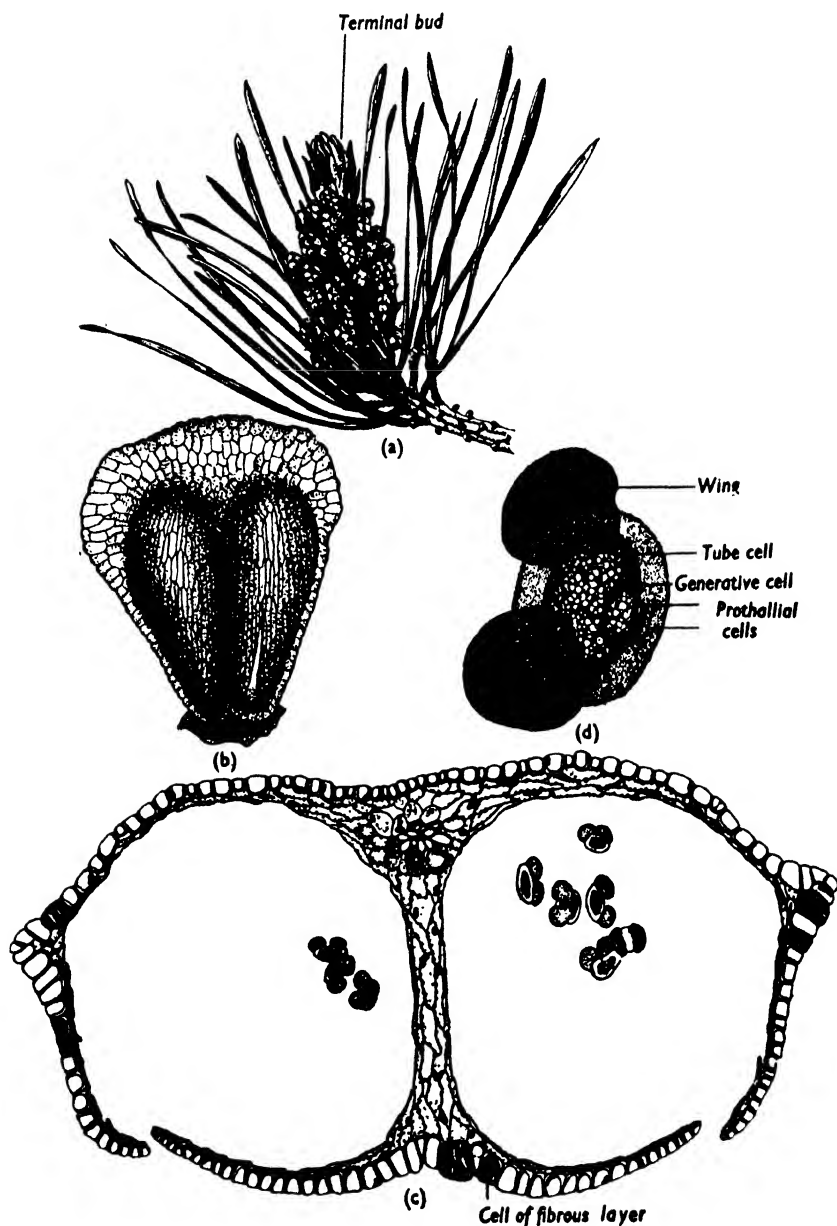


FIG. 150.—*Pinus*, (a) twig with staminate cones ($\times 1$), (b) microsporophyll seen from the lower side ($\times 16$), (c) microsporophyll in transverse section ($\times 65$), (d) mature pollen grain mounted in acetocarmine and hydrochloric acid ($\times 475$).

are flagellated sperms, in all respects comparable with those of the types just considered, and have to swim a short distance to reach the ovum.

These gymnosperms, with motile sperms, afford a useful connecting-link between the ferns and the types of gymnosperm, such as the Scots pine (*Pinus sylvestris* L.), with which we are more familiar in this country. The construction of the vegetative parts of this plant are peculiar, and not general for gymnosperms, for, except in the first year, the leaves borne on the new extension growth are small, brown scale leaves, and all the green, needle-like leaves of the plant are born in pairs, surrounded by small scales, on bifoliar spurs in the axils of these scale leaves. There are thus in this plant two types of branch, these curious dwarf shoots as well as more normal branches, which are left as buds at the end of the year's extension growth, and the following year develop as a "whorl" of branches.

The staminate cones occur in large numbers in the axils of the small scale leaves near the base of the new shoot, and replace the bifoliar spurs in this portion of the shoot (Fig. 150a). Each cone has a number of overlapping scales, on the under surface of each of which occur two microsporangia or pollen sacs. The central tissue of each sac gives rise to tetrads of pollen grains, and when the grains separate from one another, it is seen that each one has two air-filled bladders developed in the thickness of the wall, on the side where the spores have been in contact with each other in the tetrads (Fig. 150d). These bladders have the effect of making the grains extremely light for their size, and if a pine tree is shaken in June, clouds of pollen will be seen to rise into the air.

The ovulate cones are fewer in number, and are found towards the end of the new shoots, in positions corresponding with those of the buds which grow out the next year into long branches. At first each cone is pinkish in colour and stands erect on a short stem (Fig. 151a). If such a cone is dissected in June, it is found to be constructed of a number of double scales, each built up of a small scale with a thicker scale close above it. On the upper surface of each thick scale occur two ovules, with their micropyles directed obliquely downwards towards the axis of the cone, and with the single integument

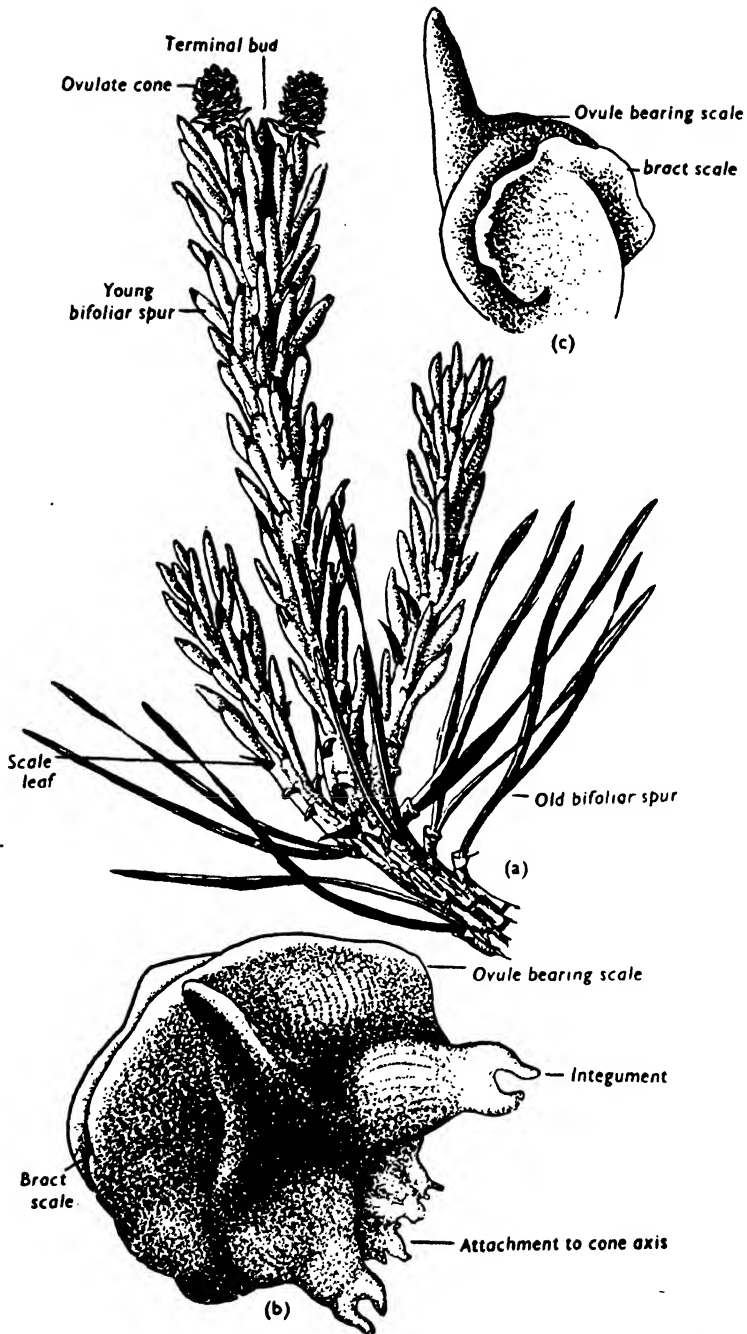


FIG. 151.—*Pinus*, (a) ovulate cones in the first year ($\times 1$), (b) two ovules on the adaxial surface of a scale, showing the splayed integument ($\times 25$), (c) a smaller double scale seen from below showing its double nature ($\times 25$).

splayed so as to leave a wide aperture down to the nucellus (Fig. 151*b*). At this stage the scales of the cone do not fit tightly over one another, and there is ready access for pollen grains, which are trapped between the scales, and, owing to the position of the young cones, roll down the obliquely tilted scales, and come to rest in the axil of the scale, close to the micropyle of the ovule. It has long been known that at this stage, in some other conifers in which the ovules are more exposed to view, a drop of liquid is exuded from the micropyle, and in this the pollen grains are caught and are drawn into the micropyle and on to the surface of the nucellus, as the liquid dries back again. In *Pinus* Doyle has shown quite recently that a somewhat similar process occurs. The exudation of a pollination drop is a very transient phenomenon, and if pollen grains are present near the micropyle, the drop may be withdrawn again after about five minutes; further, the exudation only occurs during the early hours of the morning. As soon as the drop comes in contact with a pollen grain lying near the micropyle, the grain, being readily wettable, and so light on account of the air-filled bladders, immediately floats up through the liquid, and so reaches the nucellus, where it is left deposited when the liquid recedes. By this mechanism the grains come to lie in such a position that the part which will grow into the pollen tube is in contact with the nucellus.

Before the pollen grains have been shed, they have already germinated and have formed, by unequal divisions, two small vegetative cells and a generative cell, on the side of the grain farthest from the air bladders, whilst the tube nucleus remains in the general cytoplasm of the grain. These cells can be seen in mature pollen grains, mounted in iron acetocarmine with the addition of a drop of strong hydrochloric acid, but only if the grains lie with the wings facing downwards (Fig. 150*d*). When pollination occurs, a pollen tube immediately grows out on the side of the spore away from these cells, and penetrates the tissue of the nucellus. At this stage, however, the ovule is not yet ready for fertilisation, and the pollen tube remains in this condition during the late summer and following winter. Soon after pollination the stalk of the cone curves so that the cone becomes inverted.

By comparison with the earlier types it will be evident

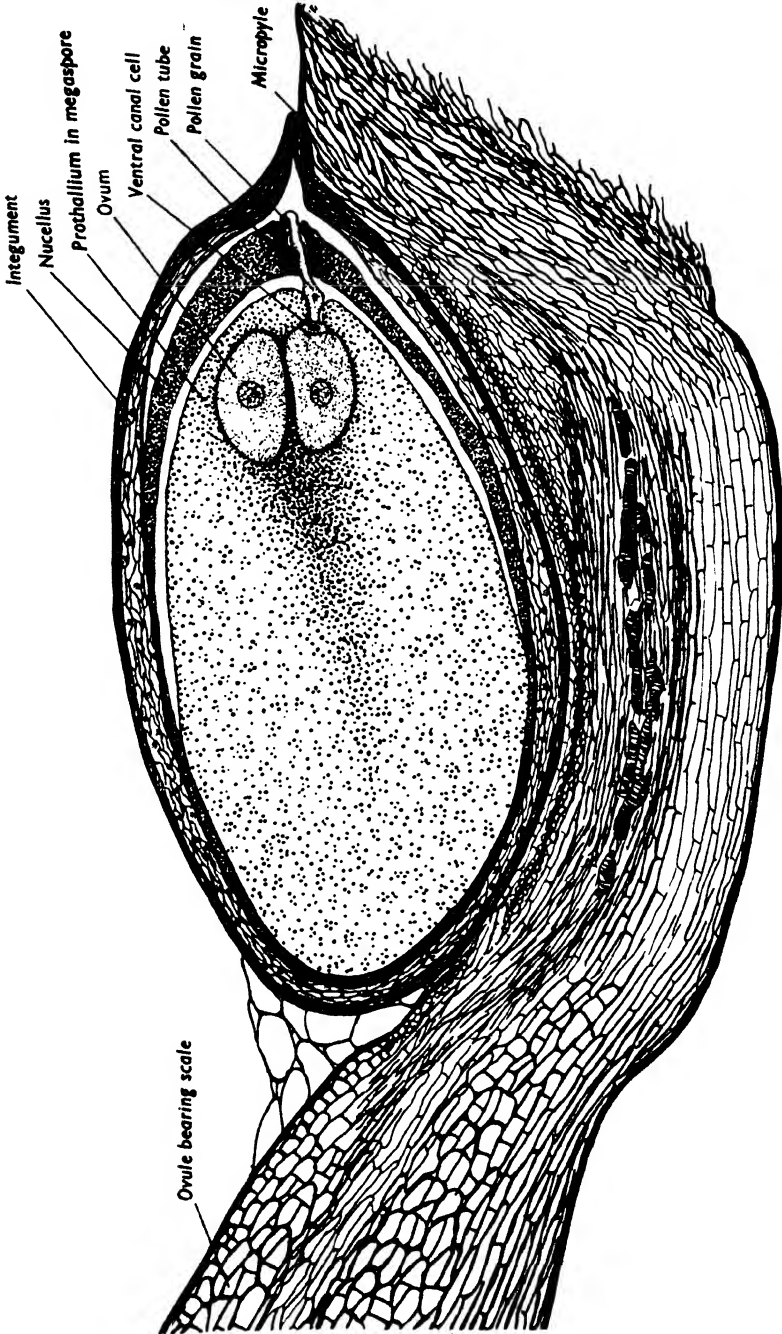


FIG. 152.—*Pinus*, section of an ovule from a second-year cone showing archegonia ($\times 32$).

that the nucellus of the ovule must be equivalent to the megasporangium, and about the time pollination takes place, a dense cell may be recognised in the nucellus, which is the megaspore mother cell. Like all spore mother cells, this undergoes two divisions in rapid succession, forming four potential megaspores, only one of which normally enlarges into the functional megaspore. By the end of the first year the megaspore has started to germinate, repeated nuclear divisions being followed by the development of walls between the nuclei; the walls appear first at the periphery of the megaspore. In the following spring this type of development continues until the megaspore is filled with tissue in which archegonia

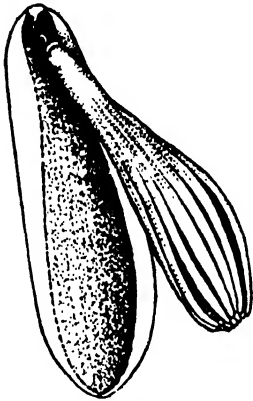


FIG. 153.—*Pinus*, embryo dissected from seed of a third-year cone of the stone pine (pine kernel) ($\times 3\frac{1}{2}$).

are developed at the micropylar end, so that this tissue, usually described as the endosperm, is evidently the female prothallium. The archegonia are fully formed by the end of June (Fig. 152). During this second spring period the male gametophyte also continues its development; the generative cell divides into a stalk and body cell, the body cell then breaks away and passes into the tube. Finally the nucleus of the body cell divides into two male nuclei, the development of which shows that they are comparable with the flagellated sperms of the cycads. These nuclei are discharged into the large egg cells of the archegonia, fertilisation takes place, and the embryo sporophyte

continues its development through late summer and autumn.

The second winter shows a change in colour of the cones from green to brown, and early in the third spring, the woody cones become dry, the scales separate from one another, and the seeds are shaken out, and are seen to have an outgrowth of the integument developed as a wing. The mature embryo may be examined conveniently in pine kernels of the stone pine (*Pinus Pinea* L.), as they are prepared for sale; the hard seed coats have been removed from these so that they are easily dissected. A layer of dense endosperm surrounds the embryo, which has a radicle at the end directed towards the micropyle, and at the other a number of needle-like cotyledons.

If the kernels are soaked in alcohol and glycerine, it is possible to show by careful dissection that the tip of the radicle is attached to a long, coiled suspensor, which, as in the angiosperms, has been developed from the part of the fertilised ovum facing the micropyle (Fig. 153).

Angiosperms.

We can now re-examine the process of fertilisation in the angiosperms in the light of this survey of the life cycles of comparatively more simply organised plants.

Evidently the angiosperm plant represents a well-developed sporophyte, and at first sight it would appear to be a heterosporous one like *Selaginella*, with pollen grains equivalent to microspores, and four cells formed in the nucellus of the ovule comparable with the four spores formed from one megaspore mother cell, but of which the embryo sac represents the one megaspore that grows to maturity. This point of view is, however, open to challenge for, at the time the divisions of the megaspore mother cell take place in the ovule, the potential spores produced are little, if any, larger than the pollen grains. The increase in size of the embryo sac is associated with those nuclear divisions and growth processes, that leave the embryo sac finally with ovum, synergidæ, definitive nucleus, and antipodal cells. The homologies of these nuclei, or cells, with the prothallial tissue in the megaspore, or embryo sac, of a pine, are by no means clear, but it is obvious that we may regard the growth processes leading to their formation as associated with the production of a female gametophyte, if of a very reduced character, rather than with the growth into a large spore. Similarly in the pine, the growth of the original spore cells in the ovule is clearly associated with the development of the female prothallium, not with the development simply of a large spore. It is, therefore, possible to argue that the original spores of the flowering plant, when they are first fully formed, a stage which is difficult to recognise in the ovule as the spores are not released from the surrounding tissue, should be regarded as equivalent in size. The flowering plant would then be homosporous like the fern, but, as in the horse-tails, the spores may be potentially different, since one gives rise to the male gametophyte, and the other to the female.

If this point of view gains support then yet another alteration in terminology is pending. It will no longer be permissible to contrast the embryo sac, as megaspore, with the pollen grains, as microspores, and further the nucellus would no longer alternatively be called the megasporangium. Alternative and more non-committal terms are indeed already proposed, viz. androspore, or male spore, for the pollen grain, and gynospore, or female spore, for the embryo sac.

In either case the tube nucleus of the angiosperm pollen grain, the tube nucleus and the prothallial cells of the gymnosperm, are all that remain to represent any part of the male gametophyte, other than the male cells. The two male nuclei formed from the generative cell will evidently be the equivalent of the sperms. As the male nuclei are carried passively to the ovum by the growth of the pollen tube, and are then discharged right into the embryo sac by the pressure of the contents of the pollen tube at the time its tip breaks down in contact with the embryo sac, the lack of flagella on the male cells is intelligible from the functional standpoint.

The carpel, the characteristic feature of the angiosperm, is a new development. Its well-developed, stigmatic surface provides a new and effective apparatus for collecting pollen, whilst the loosely packed tissue of stigma and style, surmounting the never completely closed ovary cavity, make a channel along which growth of the pollen tube is greatly facilitated. In this way the pollen tube reaches the nucellus, bringing the products of development of one spore close to the position of the other, in which a female prothallium has developed within an embryo sac. The latter has grown so as to force its way to the very apex of the nucellus, though this apex is now probably buried at the base of the narrow micropyle, formed by the incomplete closure over the sporangium of the single or double integument. The two integuments and funicle, with the details of insertion of ovules upon the placenta, are new angiospermic features, but the essential identity of nucellus with sporangium seems beyond doubt. The ovum and synergidæ may conceivably represent all that is left of the archegonial apparatus, and in that case the remaining nuclear apparatus of the embryo sac would appear to be homologues of the female prothallium.

Another new feature of the angiosperm is the remarkable

development of the endosperm that follows after the process of fertilisation, as the direct outcome of the further growth and division of the definitive nucleus after the second male nucleus has fused with it. In its formation, frequently in the early stages by nuclear divisions without associated wall formation, the angiosperm endosperm is reminiscent of the early stage of the prothallial tissue (also called endosperm) in the gymnosperm. Sometimes, therefore, the endosperm in the angiosperm is regarded as a female prothallium, delayed in development, and only formed as occasion arises for it to function as nutrient material for the still later developed embryo. However, this point of view remains speculative; we can only regard this complicated process as itself the final stage in a long and complex evolutionary history. Whilst many of its characteristics are unique and restricted to the highly organised group of the angiospermic flowering plants, the whole process of fertilisation and late endosperm development, seen against the wider background, shows most interesting parallels with, and definite indications of relationship to, the life cycles of other widely different groups of plants.

The seed, characteristic of angiosperms and gymnosperms, is now seen to be a very special structure, associated with the reduction of the gametophyte. This development itself is linked with the adoption of a land habit, and involves the nutrition of the embryo sporophyte, through the medium of the much-reduced female gametophyte, by the parent sporophyte of the previous generation. On the other hand, fertilisation, the essential process that leads to seed production, is seen to be a process that links, in essentials, the most highly organised flowering plants with the algæ, and the male cell in the pollen tube may be compared with the sperm, and ultimately with the motile stage in the life cycle of a unicellular alga.

CHAPTER XXXVII.

THE NUCLEUS AND HEREDITY.

IN Chapter VII a sharp distinction was drawn between the kind of new individuals that arise after the process of fertilisation and those produced by any process of vegetative propagation. We are now in a position to inquire into the justification for this distinction.

In vegetative propagation new individuals arise by the separation of cell aggregates, which have been formed by processes of cell growth and cell multiplication, and such individuals repeat in all essentials the characteristics of the original plant; plants multiplied in this way have been described as forming a clone. The uniformity which characterises the individuals of a clone is due to the fact that in the process of cell multiplication, at each division the organised cell contents are separated into two exactly similar daughter cells.

On the other hand, when new individuals arise as the result of a process of fertilisation, all the cells of the new individual arise from one original cell, the zygote or oospore, which has itself resulted from the fusion of two cells, or at least of two nuclei, and this individual will consequently be different from either of the parents, from which the male nucleus or the female cell were derived. The emergence of the new individual is therefore closely linked with the phenomena associated with nuclear fusion, and the appreciation of the differences between vegetative and sexual methods of propagation, involves a more complete study of nuclear organisation.

The Nucleus.

The nucleus in the living cell has already been described, and it will be realised that the analysis of the finer structure of this body is a matter for high magnifications and delicate

technique. In the living cell all that can be readily seen is that the nucleus is spherical, as would be an immiscible drop in a liquid medium, and also that it is more highly refractive than the surrounding cytoplasm. From its behaviour to stains and microchemical reagents, it is to be concluded that it is probably largely protein in nature, though these proteins are thought to be particularly complex and are described as nucleo-proteins or compounds of proteins with nucleic acid.

In fixed and stained material some constituent of the nucleus is seen to have combined readily with basic dyes, such as safranin so that the nucleus appears especially prominent. The substance which combines with the stain has received the general name of chromatin, though many different substances may be included under this term ; probably the staining reaction is mainly due to nucleo-protein, in which the nucleic acid gives the substance acidic properties, so that it combines with basic dyes. When a nucleus is not dividing, when it has with doubtful justice been described as in a "resting" phase, it is clearly separated from the cytoplasm by a fine surface film, the nuclear membrane, and within this the chromatin, in fixed and stained material, has a granular appearance. In this stage the nucleus is probably in its most active phase, growing and building up more material like itself, for in any actively growing tissue, this phase is followed by the division of the nucleus into two in the process of karyokinesis or mitosis. Each nucleus will then grow during the next "resting" phase, better termed an "interphase," to the full size again in preparation for the next division.

Karyokinesis or Mitosis.

An indication of the commencement of nuclear division is the appearance in the nucleus of a thread-like arrangement of the chromatin, in place of the granular appearance seen in the interphase (Fig. 154). The threads soon become conspicuous and are seen to lie in a loose spiral just within the nuclear membrane ; this stage is called the prophase. Up to this stage there are also visible one or more deeply staining bodies, the nucleoli, but as division proceeds beyond the prophase in most plant species the nucleoli gradually disappear, and also the nuclear membrane. As the division proceeds the prophase threads undergo considerable coiling and shortening

and finally become clearly recognisable as separate lengths of deeply staining material known as chromosomes. In any species of plant or animal the number and shape of the chromosomes which appear in successive cell divisions during vegetative growth is constant and characteristic of the species. The chromosomes have been the subject of intensive study, and it will only be possible to indicate very briefly the complexity and significance of some of the phenomena in which they are concerned.

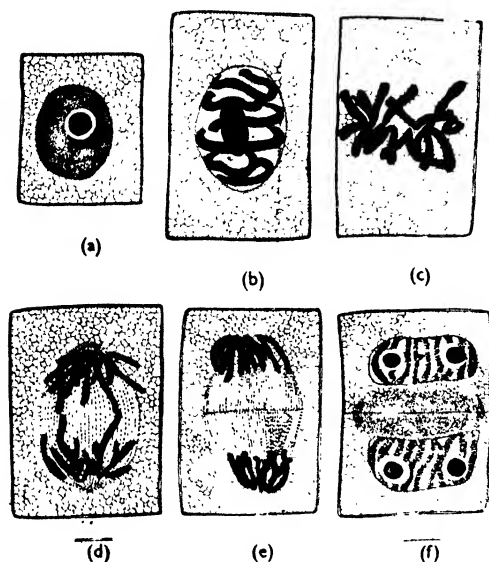


FIG. 154.—Stages in nuclear division in the root tip of onion ($\times 1350$), (a) interphase, (b) prophase, (c) metaphase, (d) anaphase, (e) early telophase, (f) late telophase.

since chromosomes have been seen when cell divisions have been observed in living cells, as in *Spirogyra* filaments or in the young hairs on the stamens of *Tradescantia*. There is evidence that at this stage the cell contents, including the nucleus, become relatively more solid, and the more viscous material composing the chromosomes may be deformed by the more solid matrix in which they lie. The central part of the cell, formerly occupied by the nucleus, becomes more homogeneous in appearance and frequently there appear in it, in fixed material, striations which radiate to two "poles"

of the cell. The chromosomes now come to lie across the central region of this "spindle," arranging themselves in the equatorial plane so far as their length will allow (Fig. 154c). The striations are sometimes described as the spindle threads, but this terminology conveys perhaps more than is known, as it is possible that the threads may be no more than evidence of strains in the protoplasmic matrix. Some of the "threads" are evidently closely associated with individual chromosomes, whilst others traverse the whole length of the spindle from pole to pole.

The formation of the spindle may be evidence that the protoplasm, as the cell divides, passes temporarily from the state of a sol to a gel. The chromosomes also are relatively solid structures, which have themselves a considerable degree of complexity. Recent work has shown that the staining material of the chromosome has a spiral construction, and the appearance of the prophase threads and their continued shortening and increase in density may be due to the progressively closer coiling of the turns of the spiral. If dividing cells from living material are exposed to ammonia under certain conditions, and then mounted in iron acetocarmine solution, which stains the chromatin, the ammonia has had the effect of loosening the spiral, which may then be seen more clearly. If the structure is truly to be regarded as a spiral, then some thread-like structure must have been present originally to have been built up into the spiral, and the obvious

suggestion is the thread built up of $\begin{array}{c} | & | & | \\ -C- & N- & C- \\ | & | & | \end{array}$ linkages,

so characteristic of the proteins (Chapter XVIII). Such a protein thread would itself be of too fine a structure to be visible under the microscope, but there is further evidence that the visible spiral is composed of a thread, which is itself coiled in a spiral of smaller diameter, so that the ultimate structure is perhaps a "cork-screw of cork-screws." Such points as these are still under discussion, but they at least indicate the complexity of chromosome structure.

The stage when the chromosomes are arranged on the spindle, in the equatorial plane of the cell, is described as metaphase, and as this condition only persists for a short time, only a few minutes as a rule, true metaphase figures are not

frequent. Each chromosome then becomes separated longitudinally into two exactly similar halves, and the two sets of chromosomes move apart from each other to the opposite poles of the spindle. The main feature of this complicated process of nuclear division is a sorting out of the nuclear organisation into a set of chromosomes, in such a way that the whole structure may be equally shared between two daughter cells by the longitudinal division of each constituent chromosome into equal halves. All the preliminary processes of the division are spoken of as prophase, the separation into two halves occurs at metaphase, and the journey of the divided chromosomes to the poles occurs during anaphase, to culminate in the telophase, when the two daughter nuclei are reorganised and gradually resume the liquid state typical of interphase nuclei.

Mitosis has been followed in a large number of different species, and though the details of structure and performance show some variation, the main features of the process are constant throughout, and the stages corresponding to those figured for *Allium* may be identified. A striking feature of the process is the amazing constancy of the chromosome sets which emerge every time that a vegetative nucleus divides; not only has every species its characteristic number of chromosomes, but each chromosome has also a characteristic size and form, and a pre-determined point at which it appears to be attached to the "spindle fibre." When two divisions follow one another rapidly, the chromosomes may retain their form sufficiently for it to be recognised that the same chromosomes appear in the same position in the prophase of the second division as that which they occupied at the telophase of the first; the extreme constancy of the chromosome sets strongly suggests that the chromosomes retain their individuality through the interphase, so that the same chromatin material is aggregated into similar chromosomes at each succeeding division.

It is important to emphasise that this process of mitosis means the exact bipartition of the nuclear substance at each division, so that every cell of a tissue or plant which has developed by the processes of cell growth and vegetative cell division, will have exactly the same nuclear constitution. This is obviously the explanation of the fact that individuals which are multiplied by the separation of parts of vegetative

tissues are all similar in their characteristics, and are the individuals of a clone. That growth of the nucleus should mean the multiplication of organisations of identical pattern is perhaps not so astonishing, since growth means that living material takes up nutrient substance and converts it into itself. In this case nucleo-proteins in a complex matrix take up more organic food and build up from it more of the same nucleo-proteins in an identical matrix. The result is that when the chromosomes are once more separated out from the interphase nucleus, each one is no longer a single structure, a fact that is sometimes recognisable even at the earliest spireme stages ; evidently the daughter chromosomes, which separate from one another at metaphase, then pass into a period of growth during which they become duplicated in preparation for the next division.

Meiosis.

In the life cycles of all types of plants we have seen that normally a process of fusion of gametes or fertilisation takes place. When this is examined in detail with regard to the nuclear behaviour, it is seen that the two nuclei coalesce and the chromosomes of both pass on to a common spindle at the next division, when each one separates into two halves as in a normal mitotic division ; by the process of nuclear fusion, however, the chromatin material and the number of chromosomes has been doubled. Thus if there were no corresponding process to halve the number of chromosomes, this number would be doubled at each successive process of fertilisation and soon become excessively large. The compensating process is associated with the nuclear divisions that occur in the spore mother cells ; in the angiosperm this means the two rapidly occurring divisions in the pollen mother cells and, in the ovule, either the two divisions which give rise to the four cells, one of which grows into the embryo sac, as in *Polygonum* (Chapter XXXIV), or the first two nuclear divisions in the embryo sac itself, as in *Lilium*.

At this point in the life cycle, two nuclear divisions follow one another very rapidly without any true interphase between them, and the first of these is a division of a somewhat different type, which seems to be interpolated between two normal

divisions. In this first division the prophase threads appear single at first, but are then seen to pair laterally with similar threads, so that the metaphase "chromosomes" are at least double structures, and as the members of these pairs separate to pass to different poles, the chromosome number is halved. Before this division is far advanced, however, the individual chromosomes also undergo their normal splitting and the nuclei immediately pass into a second division in which this

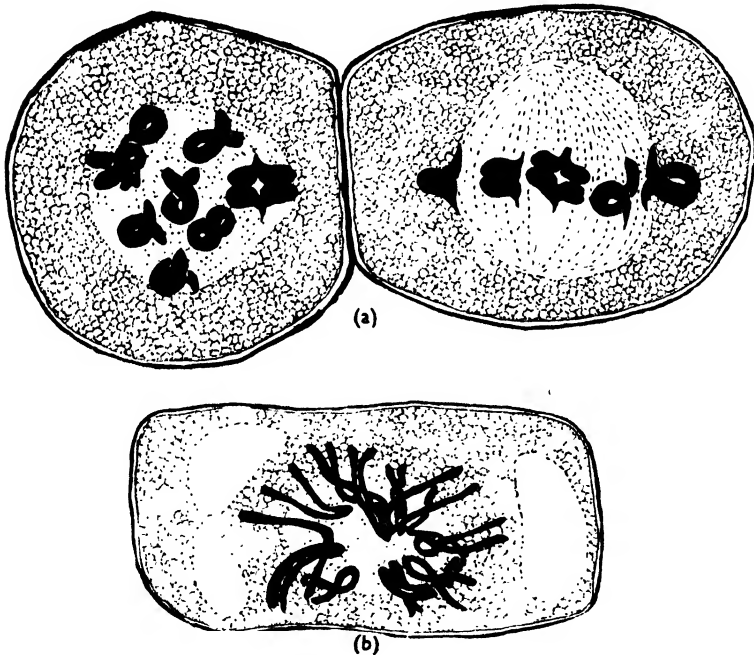


FIG. 155.—*Alstrameria*, (a) two views of meiotic metaphase in the pollen mother cells, (b) metaphase of a mitotic division in a cell of the anther wall for comparison ($\times 1900$).

separation of the chromosomes into halves becomes effective. From these two rapidly succeeding divisions four nuclei result, each with half the original number of chromosomes; frequently this number is spoken of as the n , or haploid number, as compared with the $2n$, or diploid number, formed in normal mitotic divisions. The two divisions occurring in this way in the spore mother cells, and resulting in the formation of the spores, are described as meiosis, whilst the first of these, which actually reduces the number of chromosomes to half, justifies

the use of the term reduction division. A striking feature of the first meiotic division is the much more contracted form of the chromosomes. This is particularly evident when the paired chromosomes are grouped upon the equatorial plate (Fig. 155), when they are seen as much shorter and thicker structures than the metaphase chromosomes in a vegetative division in the same species.

If the chromosome number is doubled at fertilisation, the chromosomes in the nucleus of the vegetative plant must be present in an even number ; in the great majority of cases this is true. Also it is clear that the chromosomes which pass on to the equatorial plate of the first meiotic division are typically arranged in pairs of similar chromosomes and that one of each pair goes to each pole. In this way each characteristic chromosome is represented in the reduced set which passes to each daughter nucleus, and the vegetative "diploid" nucleus must have contained two complete sets. From another aspect also this must be true, for the chromosomes evidently carry the hereditary characters of the individual. If a complete set were not separated to either pole, then when these sets subsequently fuse with other sets, similarly segregated from other meiotic divisions, certain essential characters of the original plant might be missing from both sets. The resultant fusion cell would in that case not contain a complete set of genetic characters and the fusion nucleus in the oospore would fail to reproduce the assemblage of characters by which the species is identified, or more probably would fail to develop at all.

Mendelism.

The full significance of the meiotic division, and the subsequent nuclear fusion of the gametes to give a new individual, can be better understood after a digression from cytology (the study of the cell). The full story of the nuclear cycle was first outlined when van Beneden, in 1883, showed in an animal that the egg and sperm contain half the number of chromosomes found in somatic cells, and this was confirmed for plants by Strasburger in 1888. A great observer and plant breeder, the Abbé Mendel (1822-1884), had previously discovered some facts about the inheritance of characters which

showed the necessity for some such machinery of cell division and cell fusion.

Before the time of Mendel, attempts had already been made to study the way in which characters are transmitted from one generation to another, and the greater success of Mendel's experiments was due to the fact that he did not attempt to follow the totality of characters, but, after analysing his plant types, he followed through the succeeding generations only certain specific characters; his experiments were designed as cross-pollination experiments between plants in which specific characters showed characteristic differences, e.g. in height, in pod colour, and the fate of these differing features was then followed through several generations. Mendel used garden peas in his experiments, and in this species (*Pisum sativum* L.), if no special precautions are taken, pollen of the flower falls on the stigma before the flower opens, so that peas are normally self-fertilised. Before commencing his crossing experiments, he selected certain races of garden peas differing from one another in easily observed features, and grew these for some years in the monastery garden so as to be sure that, so long as the flowers were self-pollinated, these particular features bred true. He then took these plants and cross-pollinated different races, selecting the following seven pairs of characters:—

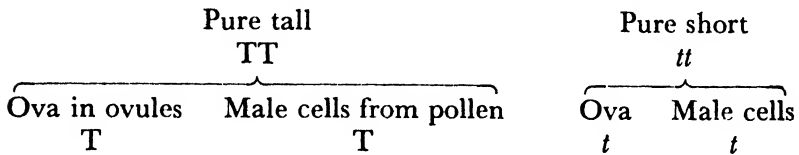
1. Height; whether tall (6 feet), or short ($1\frac{1}{2}$ feet).
2. Arrangement of flowers on the stem; whether distributed along the axis of the plant, or bunched together at the top.
3. Colour of unripe pod; green, or yellow.
4. Shape of pod; whether simply inflated, or deeply constricted between the seeds.
5. Colour of testa; whether coloured (grey, brown, or violet spotted), or white.
6. Colour of cotyledons; whether yellow, or green.
7. Shape of seeds; whether smooth and round, or wrinkled.

Having found that the tall race always produced plants approximately 6 feet high and the short race similarly produced plants constantly about $1\frac{1}{2}$ feet high, he cut out the stamens from flower buds of the tall plant and pollinated the

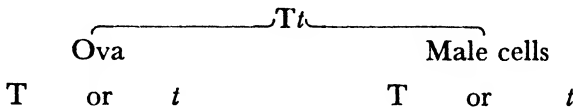
stigmas artificially with pollen from the bud of a short plant. The seeds from these plants were grown, and it was found that in this first filial generation (F_1), all the plants were consistently tall, if anything taller than the original tall type. The flowers from these hybrid plants were allowed to self-pollinate, and the seeds were planted to give the F_2 generation of plants. In this population both tall and short plants appeared, and amongst the 1064 plants grown, the proportion of tall plants to short worked out at approximately 3 tall to 1 short. This result brought out the first point of interest; although the plants in F_1 , resulting directly from the cross, were all tall, in the next generation, without the introduction of any pollen from outside, short plants appeared in the progeny. The F_1 plants must have carried in their constitution the potentialities to produce tall or short plants, but when these different determiners, or genes, which influence the height to which a plant will grow, are both present together in the same organism, one apparently has a stronger influence than the other, so that all the hybrid plants are like one parent or the other, in the present case all tall. More rarely the hybrid is intermediate in form, but the more general condition in a hybrid is for one of the genes to be completely dominant and the other recessive, when both are present together. Pairs of genes which influence the same character in the plant, as in the seven cases cited, are described as allelomorphic pairs, and in this list the dominant member of the pair is given first in each case.

When the progeny is followed to the F_3 generation, it is found that if the crops of seeds from individual F_2 plants are grown separately, from $\frac{1}{3}$ of the tall plants the seeds give only tall plants, from $\frac{2}{3}$ tall and short plants again appear in the proportion of 3 tall to 1 short, as in the case of the progeny from the F_1 tall plants, whilst the short F_2 plants give only short plants again. These proportions of dominant to recessive types were so consistent in Mendel's experiments, whatever single allelomorphic pair of genes he considered, that he came to the conclusion that when gametes are formed from a hybrid plant, the genes of the allelomorphic pair must become separated again, so that each gamete will carry only one member of the pair, in this case either the gene for tallness or shortness, but never both. It may readily be seen that

this conclusion would account for the proportions obtained ; the results may be shown diagrammatically, indicating the dominant gene for height as T and the recessive as *t*.



Cross-pollination, using either plant as the ovule parent or pollen parent, will give the same result, an F₁ hybrid plant T*t*. From this plant gametes will arise as follows :—



Gametes of these two constitutions will arise in equal numbers and, if self-pollination takes place, it is a matter of chance whether the ovule with a T ovum is fertilised by a pollen tube carrying T or *t* male cells, and similarly the *t* ovule may be fertilised by a male cell of either type. Random meeting of gametes will thus give zygotes in the proportion 1TT : 2T*t* : 1*tt*, but, since T is dominant, the progeny of this generation will appear to be 3 tall to 1 short. The difference between the TT and the T*t* individuals will only appear on further breeding, when the TT on self-pollination will only give tall plants and the T*t* will again give tall and short in the 3 to 1 ratio.

Mendel extended the experiments to cases where the crossed plants differed in more than one pair of allelomorphous characters, e.g. a tall plant with a green pod colour (G) might be crossed with a short plant with yellow pods (*g*), when the constitution of the F₁ hybrid would be T*t*G*g*. Owing to the dominance of T and G, this plant would be tall and have green pods. At gamete formation the members of the allelomorphous pairs are separated, so that T will pass to one gamete and *t* to another and similarly G and *g* will be separated, but every gamete will contain a gene determining height and one determining colour. It was clear from Mendel's numerical results from such a cross that the members of the two allelomorphous pairs segregated independently from one another,

and did not remain in the combination in which they entered the zygote from the original parents. Thus when only one pair of characters was considered, male or female gametes of the constitution T or *t* were formed; when two pairs are considered, gametes containing T will also contain G or *g*, and similarly those with *t* will also contain G or *g*. The possible gametes, on the male or female side, will be TG, T*g*, *t*G, *tg*. The simplest way to illustrate this result is by means of a chess-board diagram, in which the possible male gametes are written in one direction, the possible female gametes in the direction at right angles, and the constitution of the zygote resulting from the chance meeting of any two gametes may be read in the appropriate square.

Female gametes.	Male gametes.			
	TG.	T <i>g</i> .	<i>t</i> G.	<i>tg</i> .
TG	TGTG	T <i>g</i> TG	<i>t</i> GTG	<i>tg</i> TG
T <i>g</i>	TGT <i>g</i>	T <i>g</i> T <i>g</i>	<i>t</i> GT <i>g</i>	<i>tg</i> T <i>g</i>
<i>t</i> G	TG <i>t</i> G	T <i>g</i> <i>t</i> G	<i>t</i> G <i>t</i> G	<i>tg</i> <i>t</i> G
<i>tg</i>	TG <i>tg</i>	T <i>g</i> <i>tg</i>	<i>t</i> G <i>tg</i>	<i>tg</i> <i>tg</i>

Analysis of this table will show that out of the sixteen individuals representing the result of random meeting, the relative proportions of types appearing in F₂ will be 9 tall plants with green pods (containing T and G), 3 tall plants with yellow pods (T and *g*), 3 short plants with green pods (*t* and G) and 1 short plant with yellow pods (*t* and *g*). Of this assemblage only the TGTG and *tgtg* plants will breed true and be like the original parents, the T*g*T*g* and *t*G*t*G plants will also breed true but have a new combination of characters as compared with the parents, whilst the remainder are hybrid for one or both characters, the progeny upon selfing could again be worked out on the chess-board diagram.

The constitution of a hybrid plant may be tested in the above manner by selfing, but a more reliable method, and one for which rather smaller numbers may suffice, is to back

cross the hybrid with a pure recessive type. For example, to test the constitution of a Tt hybrid, it could be crossed with a tt type. Taking the Tt plant as the ovule parent (though obviously the result of the reciprocal cross with the Tt plant as the male parent would give precisely the same result), half the ovules will have T ova and half t . The recessive male parent can only form t pollen grains, so that on chance mating half the progeny will be Tt (hybrid dominants), and half tt (pure recessives). In a similar manner, using the chess-board diagram if necessary, it may be shown that if a plant hybrid for two allelomorphic pairs ($TtGg$) is crossed with a pure recessive for these same genes ($ttgg$), of the possible sixteen matings, one quarter will be of each of the four types, $TGtg$, $Tgtg$, $tGtg$, and $tggt$.

The results of Mendel's breeding experiments on peas were published in 1865, in the proceedings of a natural history society in Brünn, where they remained buried in the literature until their significance was realised almost simultaneously in 1900 by three independent workers, de Vries, Correns, and Tschermak. As the reduction division had been discovered about ten years earlier, the significance of Mendel's experiments was at once evident, and many breeding experiments were carried out to confirm and extend Mendel's results, in this country especially by Bateson.

In the light of these observations upon the fate of individual characters in breeding experiments, we may now re-examine the process of meiosis and nuclear fusion. Every time an ovum in an ovule is fertilised by a male nucleus, two complete sets of chromosomes, each carrying a complete set of genes for the plant species, are brought together as the $2n$ chromosome set of the fusion nucleus. These sets need not be identical, for one may tend to produce a taller plant than the other or a pod of a different colour, but the difference between the two must not be greater than can be covered by the description of the species. When two different genes affecting the same feature of the plant are combined in the same oospore, the resulting plant may show the result of the presence of only one of these, the dominant gene, though in some cases the hybrid may be intermediate in type. But the recessive gene in the first case is not permanently suppressed, nor are the genes blended in the second, for further breeding

shows that, at the next reduction division, these paired genes, borne on corresponding chromosomes from the two parents, are separated to different poles of the nuclear spindle.

Thus, in the reduction division, the gathering of the chromosomes on to the equatorial plane is an orderly process, in which corresponding chromosomes must be grouped together in pairs, and one from each pair will pass to either pole. Thus these different genes, affecting the same character, are once more separated, and if in the next fusion they are associated with a gene identical with themselves, the plant will be like one or other of its original parents with regard to this particular feature. We can thus build up a picture of inheritance; a species is constituted of a number of different genes, complete sets of which make up the individual. But since pairs of slightly differing genes are usually associated in the growing cells, we must not expect the analysis of heritable characters to be too sharp or their estimation and recognition too quantitative.

Another extremely important point concerning the behaviour of genes may be drawn from the Mendelian experiments in which the fate of more than one pair of genes was considered. Evidently the genes of different pairs separated quite independently of one another when the hybrid formed gametes. So it was found that if a tall plant with green pods was crossed with a short plant with yellow pods, after self-fertilisation, the F₂ generation included plants with all possible combinations of tall or short plants with green or yellow pods. This means that if we consider two chromosomes of a set, chromosome A carrying a gene for tallness and B carrying a gene for green pod, and this set at fertilisation meets a different set with chromosome *a* carrying the gene for shortness and *b* carrying that for yellow pod, these are associated through vegetative growth, but when gametes are formed and the $2n$ set is reduced again to two n sets, the chromosomes A and B, which were introduced from one parent, need not necessarily pass to the same pole. Any combination, AB, *Ab*, *aB*, or *ab* may occur, but it is essential that each letter should be represented in each reduced set—there must be a complete set of chromosomes separating to each pole. A good analogy is provided by a set of chessmen. Any complete set must contain king, queen, knight, bishop, castle, and pawn, and

the black and white sets may be grouped together to represent the fusion nucleus ; we can picture reduction division if we separate them once more into two complete single sets but neglect their colour. This analogy may even be carried a little further. In the vegetative tissues of the pea there are fourteen chromosomes, and each complete set after the reduction division contains seven. Breeding experiments have left no doubt, however, that there are far more than seven allelomorphous pairs of heritable qualities in peas, and that these segregate and recombine in the typical manner as shown by Mendel for single pairs. This means that each chromosome must carry a large number of genes, which we should therefore expect to fall into groups, equal in number to the chromosomes. Though genes carried on the same chromosome do not show the same degree of independent segregation as genes carried on different chromosomes, it is still found that they do not necessarily remain in the same associations as in the parent types. The genes are arranged in orderly succession along the chromosomes, and when two corresponding chromosomes are closely paired side by side in meiosis, their parts are interchangeable, and indeed must have frequently interchanged.

It is as if our chessmen, originally all white or all black, were built up of definite units along their length. After the game, as they lie together in the box, many of these units have been interchanged, so that though we can still sort out two complete sets, each with king, queen, knight, etc., each with its original shape, each one is now parti-coloured black and white. Though we have two complete sets of chessmen, their constructional units are now so inextricably mingled that it is no longer possible to say which was the original black or white piece or, in the case of the plant, which the original maternal or paternal chromosome.

Regarded in this light, we can see, in the facts of cytology, the protoplasmic framework upon which the complex machinery of inheritance is based. The facts arrived at on the genetical side in plant breeding, and on the cytological side on the structure of protoplasm, are very complicated, and it is only very slowly that we shall attain to anything approaching a complete understanding of these complex phenomena. At the present time the study of these problems

is demanding the complete attention of an army of workers, both upon plants and animals, because the facts already revealed have shown the extraordinary importance of this type of work to human progress. For example, some of the investigations have shown that such factors as resistance, or even immunity to particular diseases, yield of crop, etc., may be determined by genes that segregate and recombine as in the Mendelian experiments, in which case it is evident that it should be possible to build up races which combine such desirable qualities. Once such a combination has been attained by suitable breeding, if continued self-pollination is possible, or if some rapid and effective method of vegetative propagation can be devised for the species, such desirable strains of cultivated plants, built up in the light of our present imperfect knowledge, can be multiplied and used for the service of mankind.

In conclusion, it may be pointed out that even this elementary examination of the nuclear and genetical phenomena associated with fertilisation makes it abundantly clear why the new individual, resulting from the union of two nuclei from different parents, is always potentially something new, as contrasted with the uniform progeny multiplied in the clone by vegetative propagation. The nucleus in any species must carry literally thousands of genetic characters (genes); the two sets that meet at the fusion of nuclei from different parents are always likely to be new combinations, and thus the process of cross pollination, followed by fertilisation, is always sending into the world new plants, since they are new combinations of the potentialities contained in the genetic complement of the species. If the plants of a seed-propagated crop are examined, it will be found that though they are sufficiently alike to be classed as one species, the different individuals show multitudinous minor variations from one another. In the facts we have been studying we see a part of the machinery which explains why, even if the species appears constantly the same, living things always contain the promise of something new.

In Chapter XVIII when we considered the $\begin{array}{c} | & | \\ -C-C- \\ | & | \end{array}$ and $\begin{array}{c} | & | & | \\ -C-N-C- \\ | & & | \end{array}$ linkages, which are woven into the structure

of the proteins, we saw how the stable nature of the chemical bonds thus portrayed explained the extraordinary permanence of living matter. The constancy of many species, which in some cases (as in the minute cells of the organism *Lagena sulcata*, belonging to the foraminifera, where the pattern on the cells can be identified from fossils) appear to have persisted unaltered from Silurian times to the present day, may find a similar explanation. We now see in the genetic mechanism

a hint how these $\begin{array}{c} | & | \\ -C & -C- \\ | & | \end{array}$ and $\begin{array}{c} | & & | \\ -C & -N & -C- \\ | & & | \end{array}$ linkages

continue to build themselves up during growth as exact replicas, so that chromosome gives rise to identical chromosome, but at the same time the chromosomes, and constituent genes, may group and regroup so as to provide continually new material for the great experiment of living.

These considerations probably have significance, as almost every living plant, at some stage in its life cycle, enters a reproductive phase which is ultimately associated with nuclear fusion and the production of new individuals. In the case of the flowering plant, many features of its floral structure seem to have significance in relation to this process of fertilisation, whilst the fruit formed subsequently has an effective organisation for the dissemination of the seeds resulting from fertilisation. A brief glance at the biology of flower and fruit from this standpoint will form the subject of the next chapter.

CHAPTER XXXVIII.

BIOLOGY OF FLOWER AND FRUIT.

The Description of a Flower.

In the majority of the flowering plants the flower itself is most conspicuous, and has naturally attracted the attention of botanist and non-botanist alike. The continued observation and study of flowers has brought out the infinite variety of form and structure which they exhibit, the range being even greater than in the case of leaves. In this comparison there is one important difference, leaves are formed throughout the life of the plant and vary from the cotyledons, through a wide range of size and form during increasing vegetative vigour, to the smaller and relatively simple bract-like organs which subtend the flowers in the reproductive region. Flowers on the other hand only appear when the reproductive stage is reached and, though the range of form is very wide when one kind of plant is compared with another, within any one species the flowers are singularly constant in character, and show nothing corresponding with the range of variation of the leaves. The result of this is that, when constant characters are being selected to serve for the identification of species, the botanist uses to a much greater extent flower and fruit characters than vegetative characters. As a secondary result it is found that the description of floral structures has a much more extensive vocabulary of technical terms, even than that dealing with the form of the vegetative plant.

The structure of the flower, and the relation of the parts to one another, is most easily described with the aid of a plan of the flower, known as the floral diagram, and a view of the flower cut in half longitudinally. In many types of inflorescence the flower is borne laterally on the axis of the

inflorescence, the peduncle, in the axil of a bract, and in the floral diagram the relative position of the flower parts to the bract and axis may usually be shown. When the flower is cut longitudinally, unless otherwise stated it is cut medianly in the antero-posterior plane, which also passes through bract and axis. The flower parts towards the axis are described as posterior, those towards the bract as anterior. If small bract-like structures are present on the flower-stalk, or pedicel, they are known as prophylls, to distinguish them from the bracts on the axis of the inflorescence. In dicotyledons two prophylls may occur, one on each flank of the pedicel, in monocotyledons usually only one, in the posterior position.

In the diagram the number of parts in any whorl may be shown and also their relation to one another, whether joined or free (cohesion) ; when parts are free or have free lobes, it is sometimes possible to represent their method of folding in the bud (*æstivation*) ; the adhesion of the stamens to the corolla, when they are epipetalous, can also be represented. The longitudinal section is most important in showing the shape of the receptacle and the relative position of insertion of calyx, corolla, and andrœcium to the gynœceum. Some of the descriptive terms may be illustrated with reference to the floral diagrams in Fig. 156.

In the buttercup (Fig. 156*a*) the inflorescence axis is terminated by the oldest flower, but lateral axes arise in the axils of bracts, and thus the position of the flower buds relative to the axis may be determined. The *æstivation* of the calyx is imbricate, two sepals overlapping their neighbours by both margins, two having their margins covered by the neighbours on both sides, whilst one overlaps by one margin, and is overlapped on the other. All the parts of the flower are inserted separately upon the receptacle, the sepals and petals are then described as polysepalous and polypetalous respectively, the stamens as free and the carpels as apocarpous. The numerous, spirally arranged stamens shed their pollen outwards, which is indicated by the outline of the anther in the diagram. The longitudinal section shows that the receptacle is conical and that the series of floral parts are borne in succession upon it, so that the carpels are inserted definitely above the other flower parts, when the flower is described as hypogynous and

the ovary as superior. These characters of the buttercup may be summed up concisely as follows :—

Calyx.	5, polysepalous, hypogynous, green.
Corolla.	5, polypetalous, hypogynous, yellow.
Andrœcium.	Indefinite and numerous, free and spirally arranged, dehiscence extrorse.
Gynœceum.	Indefinite, apocarpous, spirally arranged, superior, one ovule per carpel.

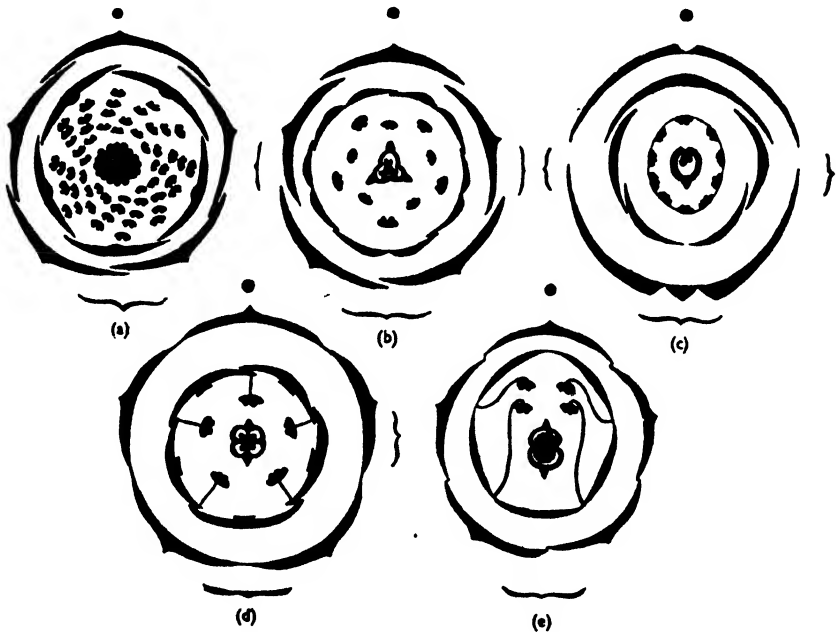


FIG. 156.—Floral diagrams, (a) *Ranunculus*, (b) *Stellaria Holostea*,¹ (c) *Ulex europæus*, (d) *Symphytum*, (e) *Digitalis*.

The same facts are also frequently represented in the form of a formula in which K = calyx, C = corolla, A = andrœcium, and G = gynœceum : $K_5, C_5, A_\infty, G_\infty$. The absence of brackets around the figure representing the number of parts indicates that they are free, and the line beneath the number that the gynœceum is superior.

In the greater stitchwort (*Stellaria Holostea* L.), the oldest flower terminates the axis but lateral flowers are borne in the axils of bracts, the flower parts are all in whorls giving the floral formula : $K_5, C_5, A_{5+5}, G_{(3)}$ (Fig. 156b). It is

¹ See footnote on next page.

usual for members of successive whorls to alternate, but in this flower two whorls of stamens are present and those of the outer whorl appear to be opposite the petals,¹ a condition known as obdiplostemonous. The three carpels are fused together into a syncarpous gynæceum with partly free central placentation.

In gorse (*Ulex europæus* L.) (Fig. 156c), two prophylls are present as two yellowish scales, one to each side of the flower. The sepals are five in number, but are fused into two groups, the number in each being indicated merely by very inconspicuous teeth at the apex, there are three in the anterior group, two in the posterior. The median petal, the standard, is posterior and larger than the others and folded over them in the bud. The filaments of the stamens are fused together at the base to form a tube; when they are all fused in this way they are described as monadelphous, but when nine of the ten are fused and the single posterior one remains free, as in clover, they are spoken of as diadelphous. The floral formula is: $K(5), C_5, A(5 + 5), G_{\underline{1}}$. In the longitudinal section of this flower, or of lupin (Fig. 160b), the receptacle is seen to be slightly flattened so that calyx, corolla, and andrœcium are no longer inserted distinctly below the gynæceum, but round about it, and the flower is distinguished as perigynous, though the gynæceum is still free and superior. Such a wide range of receptacle forms are included under perigyny, that this term often presents some difficulty to the beginner. In the lupin (Fig. 160b), the cherry (Fig. 130), and the rose (Fig. 167a) it will be seen that in all these perigynous forms the carpels are free within the receptacular cup, whilst in epigynous forms such as the daffodil (Fig. 131) or sunflower (Fig. 5a), the carpels are fused with the deeply cup-shaped receptacle, and the calyx, corolla, and andrœcium appear to be inserted on top of the inferior gynæceum.

In comfrey (*Symphytum officinale* L.) (Fig. 156d), both sepals and petals are fused to form tubes, so that the flower is gamosepalous and sympetalous (or gamopetalous). The filaments of the stamens and the corolla tube are fused in development so that the stamens appear to grow off the corolla tube (epipetalous). The two carpels lie in the median plane but are completely fused together in the region of the ovary and style, though the stigma remains bilobed. A partition divides

¹ In development the sepal stamens arise before the petal stamens, so it is more correct to describe the flower as diplostemonous.

the ovary of each carpel into two loculi, each with a single pendulous ovule.

In foxglove (*Digitalis purpurea* L.) (Fig. 156e), the sympetalous corolla is only symmetric about the antero-posterior plane and the median posterior stamen is not developed. Of the four remaining stamens, the two anterior ones have longer filaments than the two posterior and all are curved in such a way that the anthers lie against the posterior side of the corolla tube (Fig. 160a). The andrœcium of two long and two short stamens is termed didynamous, and since the stamens are also epipetalous, this feature may be indicated by the line joining the anther to the corolla tube in the floral diagram. The floral formula is : $K(5), \overline{C(5)}, A_4, G(2)$.

The attempt to interpret the diversity of form presented by flower and fruit raises again the same problems of the best line of approach, as in the case of leaf shape, discussed in Chapter XI, but in the case of reproductive structures, the attempts at interpretation up to the present have practically followed only one path. Flowers are associated with the process of pollination, and pollination is followed by fertilisation and seed production. The essential nature of these processes has been learnt in the previous chapter, and in the light of these facts it seems very natural that the explanation of flower form should have been sought in terms of its contribution to the efficiency of the pollination mechanism, whilst the fruit has been regarded rather as a structure serving first for the protection, and subsequently for the dissemination, of the seeds. Undoubtedly much light has been thrown upon flower and fruit forms by their consideration from this standpoint, which as we saw previously rests ultimately upon the assumption that structures which contribute to desirable ends, once they have arisen, have been "selected" by the increased opportunities for survival they have conferred upon the individuals bearing them. This standpoint is obviously speculative, and must be applied with caution. It is not surprising to find that in some species mechanisms are present which are efficient to prevent self-pollination, whilst in others the mechanisms render self-pollination almost inevitable. There is much yet to be learnt as to how the changed processes of development at the growing apex of the shoot have led to flower production, with which is correlated the production of germ cells, and the

initiation of the processes that lead ultimately to nuclear fusion and the creation of new individuals. It is far too early in our study of these structures to enable us to analyse the significance of the various factors that have contributed to the production of the new structures that are associated with flower and fruit. All that we can say is, that when such structures are regarded from the standpoint of their contribution to pollination mechanism, or seed dispersal, respectively, they seem to acquire much added significance; this point may be illustrated by a brief discussion of what may be called the biology of flower and fruit.

The Flower as a Pollination Mechanism.

In Chapter XXXVI we saw that the flowering plant as contrasted with other, possibly less highly evolved plants, has developed a mechanism, which is independent of any external supply of water, for the transfer of the male nucleus to the vicinity of the female nucleus. This has been accomplished by a reduction of the sexual generation, so that on the male side it may all be included within the pollen grain, which is itself so small that it can readily be carried to the sticky stigmatic surface of the carpel. In many cases the pollen grains are simply carried from the dehiscing anther to the stigma by the movements of the air, when such flowers are said to be wind-pollinated or to be anemophilous. Wind-pollinated flowers differ very markedly in both form and structure from the majority of flowers, and this difference can undoubtedly be correlated with the fact that in the larger proportion of flower types, some mechanism has been developed for pollen transfer which depends upon insect agencies, such types being known as entomophilous, or insect-pollinated.

These are the two main pollen-transfer mechanisms, though in certain water plants the pollen may be carried to the stigma by the water, and in some large tropical flowers the pollen is carried by humming birds; such flowers show undoubted correlations in structure and form with their specialised mode of pollination.

Wind-Pollination.

Although wind-pollination is exhibited by a relatively small number of species of flowering plants, as compared with those

exhibiting insect-pollination, it is readily illustrated since it is the method adopted by the majority of the ubiquitous grass family. In most of these the stem in the vegetative region remains short, but when the reproductive phase is reached, a large number of small flowers, usually in some branched inflorescence system, are carried well above the level of the foliage by the elongated axis of the inflorescence. A suitable species to examine, on account of its size, is perennial rye grass (*Lolium perenne* L.). The flowers themselves are inconspicuous, as the stamens and carpels are merely enclosed between dry chaffy scales or "glumes;" several such flowers are grouped within two sterile glumes in a "spikelet." When the stamens and stigma are mature, the glumes are forced apart by the swelling of two small structures known as lodicules, which lie on the short flower axis just above the insertion of the glumes. The filaments of the stamens are long and are attached to the anthers about mid-way along their length, so that the anthers are suspended well out beyond the glumes and readily respond to the slightest air movements. Only three stamens are developed from one flower, but as so many flowers are present in the inflorescence, the total amount of pollen is considerable. The grains themselves are smooth and light, and when the flowers are shaken they disperse as a cloud which floats in the air for a time. The ovary is surmounted by two styles, each with a feathery stigma, admirably adapted to catch the floating grains. In many cases the pollen received on the stigmas may be from the same plant, but cross-pollination is rendered more probable in most grasses by a slight difference in the time of maturity of stamens and stigmas. In foxtail grass (*Alopecurus pratensis* L.) the stigmas mature first, and it is only when these are withered and past their receptive condition that the stamen filaments elongate and the anthers dehisce. This order of maturing is called protogyny, whilst the converse order of maturing is protandry. The majority of the grasses are slightly protandrous, and with either of these two conditions cross-pollination is more likely. The grass family includes, however, wheat and barley, in which the stigmas are pollinated with the pollen of the same flower, without the flower necessarily opening at all, so that self-pollination is inevitable.

Wind-pollination is also the rule in most of those trees and

bushes which bear their flowers in deciduous inflorescences known as catkins, in which the flowers, either staminate or carpellate, are sessile in the axils of bracts along the inflorescence axis. Catkins are not always wind-pollinated, however, and it is instructive to compare the wind-pollinated trees of the genus *Populus* with the insect-pollinated trees of the genus *Salix*. Both these genera are placed in the same family, the Salicaceæ, and their comparison will indicate some of the points in the typical catkin-bearing trees which seem to have a significance in connection with wind-pollination.

In *Populus*, the staminate and carpellate flowers are borne on different trees, and in the case of some of the poplars introduced into this country the propagation has been entirely vegetative, so that all the trees of the clone bear one kind of flower only; thus the black Italian poplar (*Populus serotina* Hartig) is only found with staminate catkins, whilst the Canadian poplar (*Populus serotina* var. *aurea* Henry) only occurs with carpellate catkins.

The staminate catkins of the black Italian poplar are illustrated in Fig. 157. The long catkins emerge singly from the buds of the flowering twigs, which are formed at the extremities of the branches of well-grown trees. On such twigs a few of the distal buds remain vegetative, but below these a number of buds produce the long staminate catkins, and so much growth energy seems to be expended in their formation that these reproductive twigs make little extension growth from year to year. It is these twigs that may be abscised after many years of growth, as described and figured in Chapter X. At the base of the catkin are formed a few bud scales, whilst the rest of the long axis is covered with flowers which, at first, are hidden amongst the dark fringed bracts which subtend them. The bracts probably arise from a swollen, basal region of the catkin axis, which also bears the flowers, but they appear to arise upon the short axis of the flower itself. The flower consists of a curious expanded disc carried on a short stalk; the disc is sometimes described as the perianth, as it is the only structure present which could possibly be regarded as representing this part of the normal flower, it may, however, be more of the nature of an expanded receptacle, as upon it are borne a large number of separate stamens, with long, red anthers. There may be from eighteen

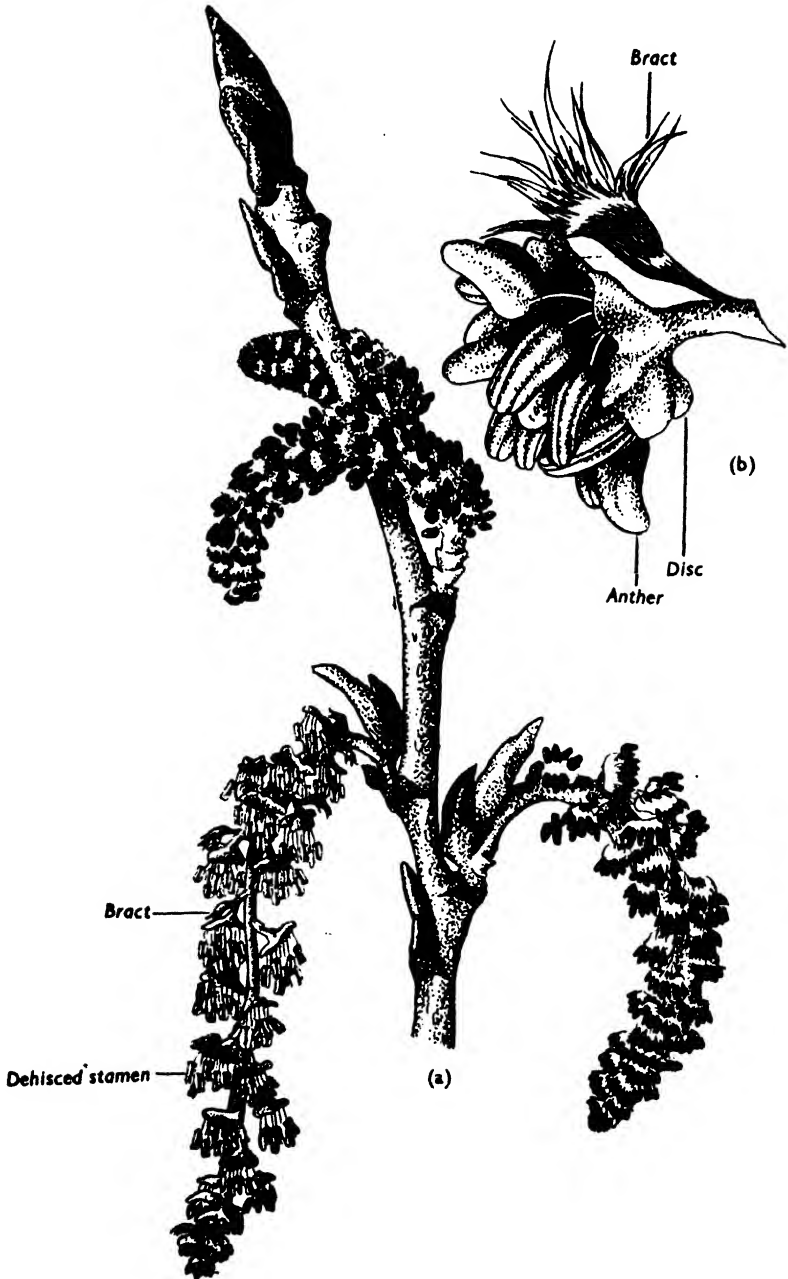


FIG. 157.—*Populus*, staminate catkins ($\times 1$), staminate flower ($\times 10$).

to twenty-four stamens in each flower, and they hang down from the cup of the disc, as this is inverted by the pendent position of the catkin. At first all the flowers are closely held together between the bracts, but at maturity the catkin axis lengthens remarkably, growing rapidly from 1 to 2 inches to a length of 3 to 4 inches; the anthers then all ripen at about the same time, and the fine white pollen is released and carried away by the wind or falls on to the backs of the lower discs to be blown away subsequently. The slightest movement of the catkins in the breeze disengages a fine cloud of pollen, which can be seen to remain suspended in the air. If some of this pollen is examined under the microscope, the rounded, smooth-coated grains are seen, but it is also evident that many of the grains are not properly developed, as in this species, owing to its hybrid origin, only a small proportion of the pollen is fertile. In the grey poplar (*Populus canescens* Sm.) the staminate catkins are very similar and a larger proportion of the pollen is fertile.

Fig. 158 represents the carpellate catkins of the balsam poplar (*Populus candicans* Aiton). Here again the flower is simply represented by a cup-like disc on which the two joined carpels are borne and in which, prior to pollination, they are nearly completely enclosed between the bracts, so that only the two-lobed stigmas are exposed. These catkins are less extended before pollination and stand more erect, and thus pollen from the air may settle upon the stigmas. After pollination the fertilised carpels swell considerably, and the catkin axis extends so that the fruiting structure is much more pendulous, a feature which at a later stage may facilitate the dispersal of the hairy seeds by the wind. The ovary has a single loculus with two parietal placentæ (Fig. 158c and d).

In Fig. 159 the staminate and carpellate catkins of sallow (*Salix Caprea* L.) are represented. Neither of these catkins has a pendulous habit, though the carpellate catkins may droop after fertilisation. The relatively stiff catkin axis will support the weight of a heavy insect such as a bee, and in the spring the bees often throng around these trees in very large numbers. Staminate and carpellate catkins are borne on different trees. The carpellate flowers consist simply of a stalked ovary, surmounted by a small forked stigma, and again unilocular with two parietal placentæ. The ovary

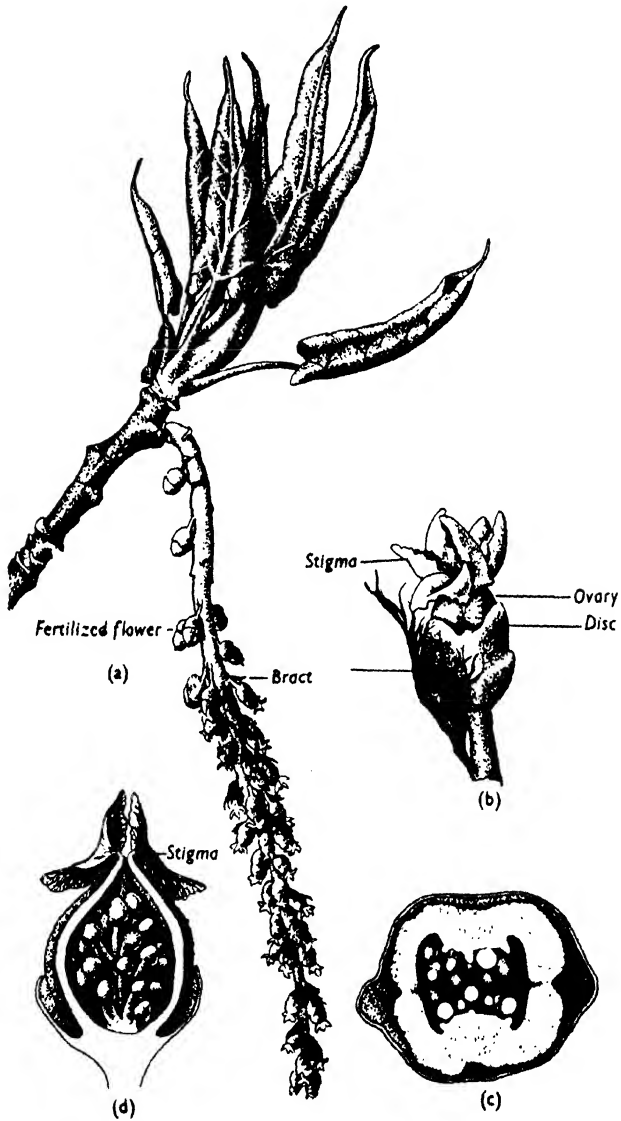


FIG. 158.—*Populus*, (a) carpellate catkin ($\times \frac{3}{2}$), (b) single flower ($\times 5$), (c) ovary in transverse and (d) in longitudinal section showing parietal placentation ($\times 10$).

stands in the axil of a hairy bract and bears in front of it a yellow-green nectary, upon which is often seen a drop

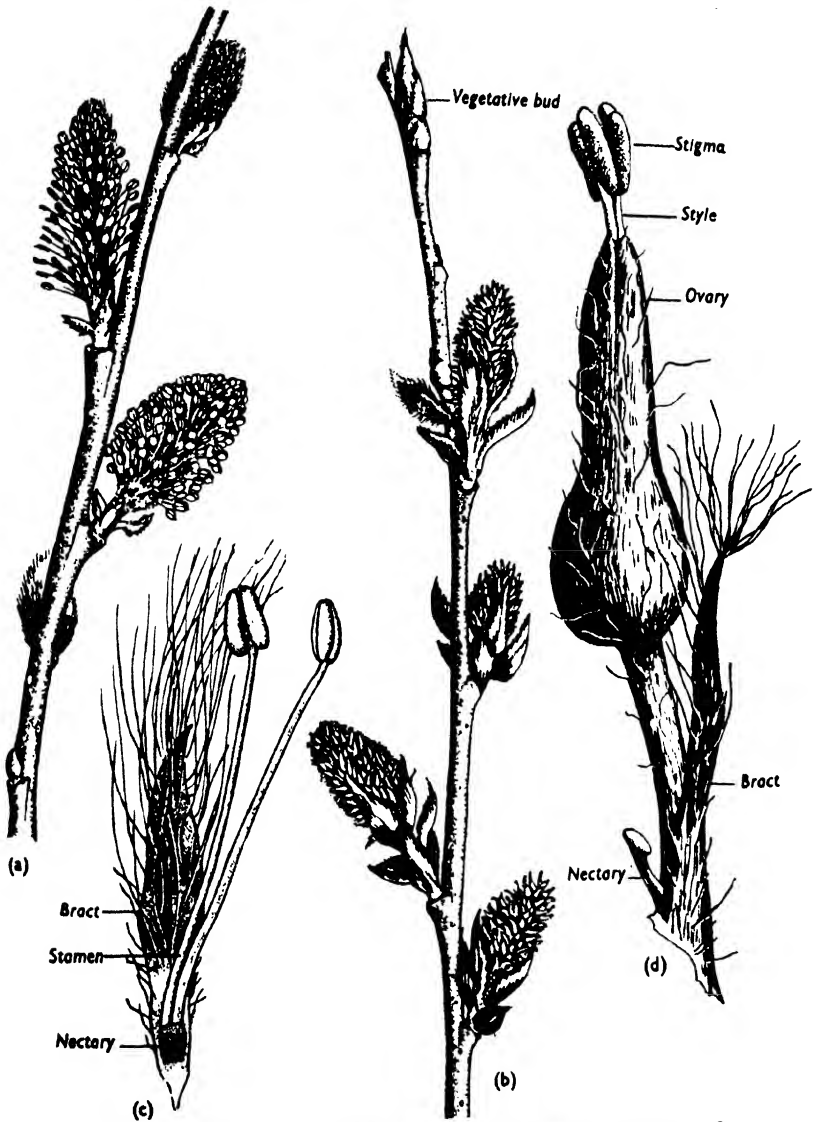


FIG. 159.—*Salix Caprea*, (a) staminate and (b) carpellate catkins ($\times \frac{1}{2}$), (c) staminate ($\times 12$), and (d) carpellate flowers ($\times 18$).

of the sugary liquid, so attractive to insect visitors. In the staminate catkins a further attraction is provided

for the visitors in the pollen itself. The flowers consist simply of two to five stamens in the axil of a hairy bract, and with a nectary standing between the stamen filaments and the catkin axis. In *Salix purpurea* L., which seems at first sight to have but one stamen, the anther head will be seen to be double, and actually the structure is the result of the complete fusion of two stamens along both filament and anther. In this species the anthers are red in colour, but in the majority of willow species they are yellow, and in either case the colour is sufficient to make the whole catkin conspicuous. When the anthers burst and release the yellow pollen the catkins become even more conspicuous as the pollen grains, unlike the poplar pollen, have a rough and sticky surface, and remain piled upon the anthers and do not blow away. The pollen is only removed when the insect visitors arrive ; much of it is collected by bees which sweep it with their fore legs into the sacs on their hind limbs, to carry away for storage in the hive for the nutrition of their larvæ. But at the same time the insect's body is dusted with the pollen, and when they visit a carpellate catkin, and scramble over its surface as they suck the nectar from the nectaries, some of the pollen is sure to come in contact with the sticky inner surfaces of the stigmatic lobes, and thus pollination is effected. Contrasting the two types, pendulous catkins, very large numbers of stamens, and release of very light dusty pollen into the air, are associated with the wind-pollination mechanism, whilst more erect catkins, a smaller number of stamens, with sticky pollen, and smaller stigmatic lobes, are associated with the pollination mechanism which relies upon insect visitors.

Insect-Pollination.

In the larger proportion of flowering plants the flowers are visited by insects, and through their agency the pollen is carried in some way from anther to stigma, but in every case the relations of insect and flower require close analysis before an interpretation is given of the inter-relation of flower structure and insect visitor. Such close analyses of specific examples is beyond the scope of this book, and therefore it must suffice to give an indication of a few general points in connection with insect-pollination mechanisms, indicating examples which may be studied as illustrations of the general statements.

Types of Insect Visitor.

In the first place there are very numerous classes of insects which by no means exhibit the same qualities as transporters of pollen. Many small insects appear to wander on to flowers in a quite fortuitous manner, possibly feeding on sugar from the nectaries or on the nutritive pollen. Owing, however, to the incidental nature of their visits, such insects may never visit the same kind of flower again, so that any pollen they may carry away with them is wasted, so far as the pollination mechanism of that particular kind of flower is concerned. Where stamens and carpels are present in the same flower, they may bring about self-pollination or, where many flowers are closely packed together as in the head of a daisy, they may transfer pollen from one flower to another on the same plant, which in all essentials is likely to have the same effect as self-pollination, but they cannot be relied upon to bring about cross-pollination between flowers on different plants of the same species. In many flowers, access of casual insects of this kind to the nectar is precluded, or rendered less likely, by various features of form and structure. The commonest of these is the development of a tubular, sympetalous corolla, which often encloses the stamens and at the base of which are situated the nectaries, as in primrose (*Primula* spp.) and comfrey (*Symphytum*), or in other cases one or more special spurs may be developed to enclose the nectary, as for example that developed from a backwardly directed petal in pansy (*Viola* spp.), or from a lobe of the tubular corolla as in toadflax (*Linaria*). In many cases the entry of small insects is made more difficult by special devices, as by the presence of scales around the mouth of the corolla in borage, or hairs at the entrance to the spurred petal in pansy, but in most of the flowers which might be regarded as having some means of protection of the nectar from small insects, it is still readily accessible to insects with longer tongues, such as bees, butterflies, and moths. Amongst the flowers which fall into this category there is very naturally some degree of gradation, so that those with a shorter corolla tube are likely to be visited by a wider range of insects, whilst in those with very long tubes, such as the red clover (*Trifolium pratense* L.), the nectar is only able to be reached by an extremely long-tongued insect like the humble-bee. This adaptation of

structure may fail sometimes to attain its object ; for example, in the runner bean the nectar is "protected" by the two lowermost petals, which are folded closely together to form what is described as the "keel." This has the same effect as the development of a corolla tube in rendering the nectar less accessible to shorter-tongued insects. It is sometimes noticed in gardens that the beans are failing to set fruit, when it may be found on examination that a short-tongued humble-bee has been robbing the flower of its nectar by biting through the base of the keel, without bringing about any pollen transfer.

Such a selective effect of form and structure upon the classes of insect visitors might well have considerable value to the flower, for some insects, and bees in particular, are very regular in their habits. Having commenced to visit flowers of a particular species, they continue for the rest of the day to visit only flowers of the same species, so that the nectar is not mixed, and pollen from different kinds of flowers is stored in separate wax cells in the hive. Evidently pollen, adhering to insects of this kind, is likely to be carried to the stigma of the same kind of plant, and cross-pollination is rendered more probable. In many such cases there are additional devices which make pollination of the stigma by pollen of the same flower almost impossible, as for example when the stamens and stigma mature at slightly different times, but where each, as they mature, assumes in turn the same position in the flower, so that an insect visiting a flower at one time will come in contact with the stigma, but at another time will have the same part of its body brought in contact with the dehiscing stamens. Two very clear cases of this kind may be selected as illustrations. The foxglove (*Digitalis purpurea* L.) is a flower with a wide tubular corolla of a purple-red colour, spotted inside with darker spots in lighter, circular patches. The anthers of the two long and two short stamens lie close against the upper side of the corolla tube. The ovary is surmounted by a long style, which is forked distally into the two stigmatic lobes, and around the base of the ovary there is a nectary in the form of a swollen ring from which nectar tends to collect at the lower side of the base of the corolla tube. The flowers are pollinated by large humble-bees. The flowers are markedly protandrous, so that the first visitors, as they press into the tube to reach

down to the nectar, have their backs dusted with the pollen from the anthers. Slightly later the two lobes of the forked stigma, now a few millimetres beyond the stamens, open out and thus expose the inner stigmatic surface, and the back of

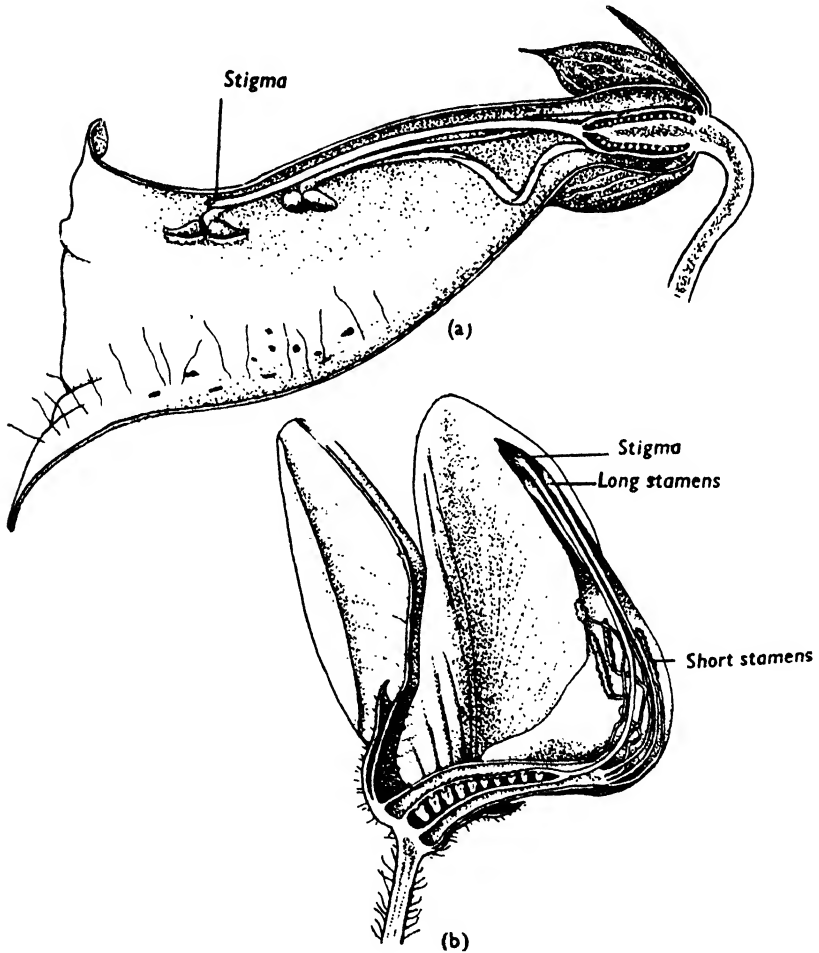


FIG. 160.—Views of flowers of (a) *Digitalis* ($\times 1\frac{1}{2}$), and (b) *Lupinus* ($\times 3$), in median longitudinal section.

any humble-bee visiting a flower at this stage comes in contact with the stigma first, and may thus transfer pollen to it from another flower (Fig. 160a). A second flower illustrating the same effect, to a less degree, is the lupin. (*Lupinus polyphyllus*

Lindl.). Like all members of the pea family (Leguminosæ), the corolla of five petals is arranged in a very characteristic way. The largest petal is posterior and erect, the two lateral ones project like two wings, whilst the two anterior and lower ones are closely interlocked to form the keel. The filaments of the ten stamens are fused into a tube in the lower part, and this surrounds the single carpel. More distally the anthers of the five longest stamens surround the stigma, which at first extends beyond the anthers. These essential parts of the flower are enclosed within the two keel petals (Fig. 160b). No nectar is secreted by lupins, but the flowers are visited by pollen-seeking bees which alight on the wing petals and, if the insect is sufficiently heavy, both these and the keel petals are depressed. During this process the stigma protrudes through the pointed end of the keel and comes in contact with the under side of the insect, where there may already be present pollen from another lupin flower previously visited. As the keel is further depressed, pollen is also squeezed out of the keel, and adheres to the insect as it presses down to obtain the pollen that has been shed into the lower part of the keel from the shorter stamens. When the insect leaves, the stigma returns to its original position, and the whole mechanism may be worked repeatedly.

Some light thus seems to be thrown upon the prevalence of gamosepaly and sympetaly by the contribution these conditions may make to effective cross-pollination. In some tropical plants the tubular corolla may reach an astonishing length, and the flowers are either visited by moths with very long probosces, or by humming birds, which hover above the flowers and thrust their long narrow beaks down to the nectar. Such visitors naturally thrust with considerable force, and plants pollinated by birds frequently have tough, and often lignified, corolla tubes, styles and staminal filaments.

Bees are comparatively heavy insect visitors, and alight upon the flower to obtain pollen or nectar, and many of the structures developed by calyx or corolla seem well adapted as alighting platforms. In the Leguminosæ, of which the lupin has been described, the arrangement of the wing and keel petals serves this purpose. In the white deadnettle (*Lamium album* L.), the lobes of the mouth of the sympetalous corolla indicate that it is constructed of five petals. The

of certain insects to nectar or pollen, which are the essential attractions of the flower to insect visitors, colour and scent probably have much significance in attracting the attention of the insect to the flower in the first place. The massing of flowers against a background renders them more conspicuous, and flower grouping, whether the result of the simultaneous blooming of the crowded plant species in an alpine meadow, or the smaller grouping of the flowers of one plant into a compact inflorescence, must have its significance. From this point of view the various types of inflorescence need some fuller consideration. When flowers are not solitary but grouped into branch systems, the resulting inflorescences are usually regarded as falling into two main systems. In the one system the flowers arise laterally in succession upon the axis of the inflorescence, the oldest being farthest from the growing point, the younger ones arising nearer and nearer to the growing apex, which may or may not ultimately be used up in flower production. Such systems are described as racemose or indefinite; the inflorescence is known as a raceme when each flower has a distinct pedicel, as a spike when the flowers are sessile. If the pedicels of the lower flowers of a raceme elongate so as to give a flat-topped inflorescence, the resulting type is a corymb (e.g. candytuft—*Iberis amara* L.), whilst if the internodes of the main axis fail to elongate between successive pedicels, an umbel is produced. In the family of the Umbelliferæ, as in keck (*Anthriscus sylvestris* Hoffm.), the system is usually more complicated forming an umbel of umbels or a compound umbel. When in a similar way the axis of a spike fails to elongate, a compact head of flowers known as a capitulum is formed, as in the Compositæ (e.g. dandelion, *Taraxacum officinale* L.). When, in a racemose system, each lateral member is not simply a flower but a branch system of flowers, a panicle is produced. The subsidiary branch systems may themselves be racemose or may belong to the cymose system now to be described.

In the second type of branch system, the definite or cymose, the first flower to develop terminates the growth of the main axis, and the further branching of the system is continued from lateral buds. If only one lateral branch, again terminated by a flower, is borne on the main axis, and the subsequent flowers similarly arise on subsidiary axes in succession

in the same way, the system is known as monochasial. The branches in such a system frequently lie in one plane ; if they appear in succession on the equivalent side of each subordinate axis, the cyme is known as helicoid, if on alternate sides in succession, as scorpioid. If below the terminal flower, two branches arise as a pair, as is commonly the case in plants with decussate phyllotaxis, and if the successive branches on the subordinate systems arise in the same way, the whole system is known as a dichasial cyme.

Considering these types of inflorescence from the standpoint of insect visitors, the flat-topped inflorescences such as the corymb, capitulum and umbel, will be conspicuous to the insect flying above the plants and these types are commonly those of open meadowland, etc. The more elongated types, such as the raceme and spike, will be more conspicuous to the insect flying beside the bush or hedgerow. It is, however, obviously open to doubt whether such considerations can, in the first place, have contributed to successful cross-pollination, and thus to the survival of the species. The crowding of flowers into a dense inflorescence, such as the capitulum, often leads to a biological specialisation amongst the flowers. In many of the Umbelliferæ, the marginal flowers are larger than those more centrally placed, and also the marginal flowers themselves are more developed on the free outer side so that they are zygomorphic. In capitula of the Compositæ, the same kind of effect goes further and affects the flower parts in such a way that the marginal flowers in some species have their corolla developed, apparently at the expense of the essential organs. Thus in daisy (*Bellis perennis* L.), the white ray florets around the margin of the inflorescence have no stamens, though the gynæceum is normal, whilst in cornflower (*Centaurea Cyanus* L.) the capitulum owes its conspicuous appearance almost entirely to the bright blue marginal florets, which are completely sterile. Though such an arrangement leads to economy of parts, since relatively few flowers render the whole inflorescence conspicuous, it seems probable that such a growth habit may rather be the consequence of spatial conditions during development, than the result of natural selection because of its added effectiveness as an attraction to insect visitors.

The significance of the mass arrangement of colour,

determined by form and inflorescence, is thus far from clear, but there can be little doubt that there is correlation between colour and scent and the type of insect visitor. Here we must guard against the interpretation of insect behaviour in the light of our own impressions of floral display. Clearly contrasting colour effects, against a background of green foliage, have to be judged in relation to the impression they make upon the eye of the insect, not upon the human eye, and of recent years this subject has been re-examined from this standpoint. A valuable résumé of this experimental work will be found in MacGregor Skene's "Biology of Flowering Plants." Von Frisch has shown that bees have a limited colour sense. They appear to be able to distinguish yellow from blue, but not red from green; their reaction to colours approximates therefore to that of a person colour blind to red and green. If this is the case, yellow, blue and purple flowers would be distinguished by bees against a green background. The behaviour of bees in the presence of a series of coloured papers was observed; they almost invariably settled on the yellow or blue strips, irrespective of the fact that they were of no greater light intensity than some of the red or grey strips. It is therefore a significant fact that flowers are frequently blue or purple, which in form are particularly suited to long-tongued insects such as bees. Yellow and white flowers are much more common, and though many are visited by bees, the nectar is often accessible to almost any type of visitor, and such flowers receive indiscriminate attention. Some flowers with very long, narrow corolla tubes are white. Usually these do not have any special part suited to serve as an alighting platform, and are open and strongly scented at night, when night-flying moths hover about them. Flowers of this type are the Nottingham catchfly (*Silene nutans* L.), and the white tobacco flower (*Nicotiana*), the white colour of which makes them relatively conspicuous at night.

Scents of flowers also show marked relation to the type of insect visitor; some unpleasantly smelling flowers, such as the wild arum (*Arum maculatum* L.) and the sickly scented hogweed (*Heracleum Sphondylium* L.), are especially attractive to flies; the ethereal oils which are attractive to ourselves seem also to be attractive to bees. The strong and more aromatic scents are associated with flowers visited by moths or butterflies, as

for example the honeysuckle (*Lonicera Periclymenum* L.), which is pollinated by moths flying at night or dusk, and the purple-flowered garden shrub, *Buddleia variabilis* Hemsl., which evidently has a strong attraction for butterflies.

It is obvious, however, that the problems opened up in the interpretation of these complex relationships between flowers and insects are too specialised and numerous even to be tabulated in this brief introductory statement. The reader must either turn to the special monographs for information if he wishes to follow up the subject, or if he wishes simply to gain experience of these problems at first hand, he cannot do better than take one or two specific examples and examine them closely. Preferably, if a copy of "Types of Floral Mechanism," by A. H. Church, is available, one or two of the flower types there described may be carefully examined in the field, and observations made whenever possible upon the nature and behaviour of any insect visitors. Comparison of these observations may then be made with the detailed records and splendid illustrations contained in that monograph. If this book is not available, a wider range of types will be found described in less detail in "Biology of Flowers" by James and Clapham.

Space will only permit of the discussion here of a very limited number of types of insect pollination mechanisms. In the first place a few examples may be considered from different genera of the buttercup family, Ranunculaceæ. In the meadow buttercup (*Ranunculus acris* L.) (Fig. 162a), the calyx consists of five free green sepals, the corolla of five free petals, which are bright yellow in colour and at the base of each of which nectar is secreted. The stamens are very numerous and spirally arranged, they mature and shed pollen in succession, commencing with the outer ones; each stamen sheds its pollen on the side away from the centre of the flower. The carpels are also relatively numerous, free from one another and each contains a single ovule. When the flower first opens, the stamens are all curved inwards and conceal the carpels, and for some time such flowers may be visited by insects for pollen or nectar, without much likelihood that pollen will be transferred to the stigmatic surfaces. At a later stage the younger stamens will also be curving away from the centre of the flower and shedding their pollen, and visiting insects will



FIG. 162.—Views of flowers in median longitudinal section, (a) *Ranunculus* ($\times 1\frac{1}{2}$), (b) *Helleborus* ($\times 1$), (c) *Delphinium* ($\times 2$).

have to crawl between the carpels and stamens to reach the nectar. It seems most probable that small insects will tend to transfer pollen to the stigmas of the same flower, though when the visitors are bees it is possible that the outward curvature of the stamens and their outward dehiscence may sometimes contribute towards cross-pollination. It is clear that this flower is not specialised for any particular type of insect visitor. The yellow colour will be perceived by bees, but there is nothing in the form of the flower to prevent other insects from reaching the nectar, and though buttercups are freely visited by insects, it seems most probable that self-pollination will be brought about more often than cross-pollination.

The Christmas rose (*Helleborus niger* L.) (Fig. 162b) is a plant introduced into English gardens from South Europe and Eastern Asia, and in this country it flowers over the period from January through early spring, when insects are scarce. The flowers are conspicuous owing to the five white sepals, the petals range from about ten to twenty, and are modified into small, slipper-shaped nectaries. The stamens are numerous and surround a group of about five carpels; the flowers are protogynous and the receptive stigmas extend beyond the stamens. Later the stamens dehisce, shedding their pollen in towards the centre of the flower, and any insect alighting on the carpels or sepals would become well dusted with pollen as it came in position to obtain nectar. This flower is not strongly specialised for pollination by any special kind of insect, as far as can be judged from its colour or form, except that the nectar, collecting at the bottom of the tubular nectaries, is not readily accessible to insects with sucking apparatus less than 2 to 3 mm. in length. In the natural habitat it is probable that bees are the most important visitors, and that these will tend to effect cross-pollination owing to the condition of protogyny. The carpels are, however, still receptive for some time after the stamens begin to dehisce, and insects of various other types visit the flowers as well as bees. The flowers set seed well when self-pollinated.

The blue garden *Delphinium* (Fig. 162c) is a specialised humble-bee flower. The sepals are bright blue in colour, and render the compound racemose inflorescence of flowers

conspicuous. The flowers are zygomorphic, and the anterior sepals present a convenient landing stage, whilst the posterior sepal is extended backwards into a long spur. The petals are relatively small and inconspicuous, but the two posterior are extended back as spurs into the spurred sepal into which they secrete nectar. The numerous stamens are spirally arranged and dehisce in succession; they surround a group of three carpels which are slightly joined. The flowers are protandrous, and as the stamens mature they curve upwards and occupy a position at the mouth of the spur; when these have all shed their pollen and have withered, the stigmatic surfaces become receptive, and these in their turn undergo curvatures and come to occupy the position at the mouth of the spur, previously occupied by the dehiscing anthers. The spur is so long that the nectar is only accessible to certain kinds of humble-bees, with probosces of at least 13 mm. in length. As the insect presses down into the spur, its head, in a young flower, becomes dusted with pollen, and if an older flower is then visited, this same part would come in contact with the stigmatic surfaces. Bees visiting an inflorescence of this kind usually start at the lower flowers and work upwards, so that in a protandrous form of this kind cross-pollination would be effected when the bee passed from the upper and younger flowers of one inflorescence, in the pollen shedding condition, to the lower and older flowers of another inflorescence, in which the stigmas would be receptive. It is frequently to be noticed that the lower flowers of a long inflorescence of this kind are the ones to set the most seed, but it is difficult to say whether this is due to cross-pollination or to the fact that they are more vigorous than the upper flowers, which often bloom late in the season.

To take one example of a flower of another family, the common primrose, *Primula vulgaris* Huds., illustrates a sym-petalous type. The calyx is tubular, and the corolla forms a long narrow tube surmounted by five free lobes. The stamens are epipetalous. The ovary lies at the bottom of the tube and the style is surmounted by a ball-like stigma; nectar is secreted from the base of the ovary and collects in the bottom of the corolla tube. There are two kinds of primrose flowers, the pin-eyed, in which the mouth of the corolla tube is occupied by the stigma, and the thrum-eyed, which are so

called because the five stamens occupy the mouth of the tube, and thus have some resemblance to a tuft of coarse threads (Fig. 163). Both types of flower are perfect, and in the pin-eyed the stamens are to be found about half-way down the tube, whilst the stigma is found in a similar position in the thrum-eyed. Primroses may be visited by various kinds of bees and humble-bees with probosces about 12 mm. in length; these alight on the corolla lobes and as they reach for the nectar, pollen may be transferred by the proboscis from a pin-eyed to a thrum-eyed flower, or by the under part of the body or the insect's head from a thrum-eyed to a pin-eyed flower. The stigma of the pin-eyed flower is coarsely papillate and suited to hold the larger pollen grains of the thrum-eyed flower, whilst the stigma of the thrum-eyed is finely papillate and thus better fitted to hold the smaller grains of the pin-eyed flower. Nevertheless, if the stigmatic surfaces of the flowers are observed in nature, usually the short-styled, finely papillate stigmatic head will show the presence of more large grains, presumably dropped upon it from its own stamens above. Such self-pollination must frequently be the rule in a cold English spring, when suitable insect visitors are scarce. On the Yorkshire coast the only insect visitor often to be recorded is a small thrips that could be but little use for cross-pollination. Some English observers are convinced that the flowers are usually self-pollinated, though others have seen both humble-bees (*Bombus* and *Anthophora*) and bee flies (*Bombylius*) visiting the flowers.

Self-pollination obviously may occur in the young thrum-eyed flower, and even in the pin-eyed flower the drooping position of the older flower may render this possible when the flower is shaken by the wind; the small thrips crawling up and down the tube may also assist self-pollination. It would seem that the English primrose must often set seed by self-pollination methods which are effective *in spite of* the striking heterostylic dimorphism which seems, at first sight, an effective device to produce cross-pollination. Charles Darwin found that the legitimate unions, in which the stigma receives pollen from a flower of the other type, gives more seed than is obtained when the stigma receives pollen only from flowers of the same type, but self-pollination may give good seed, and the evidence suggests that in some parts of

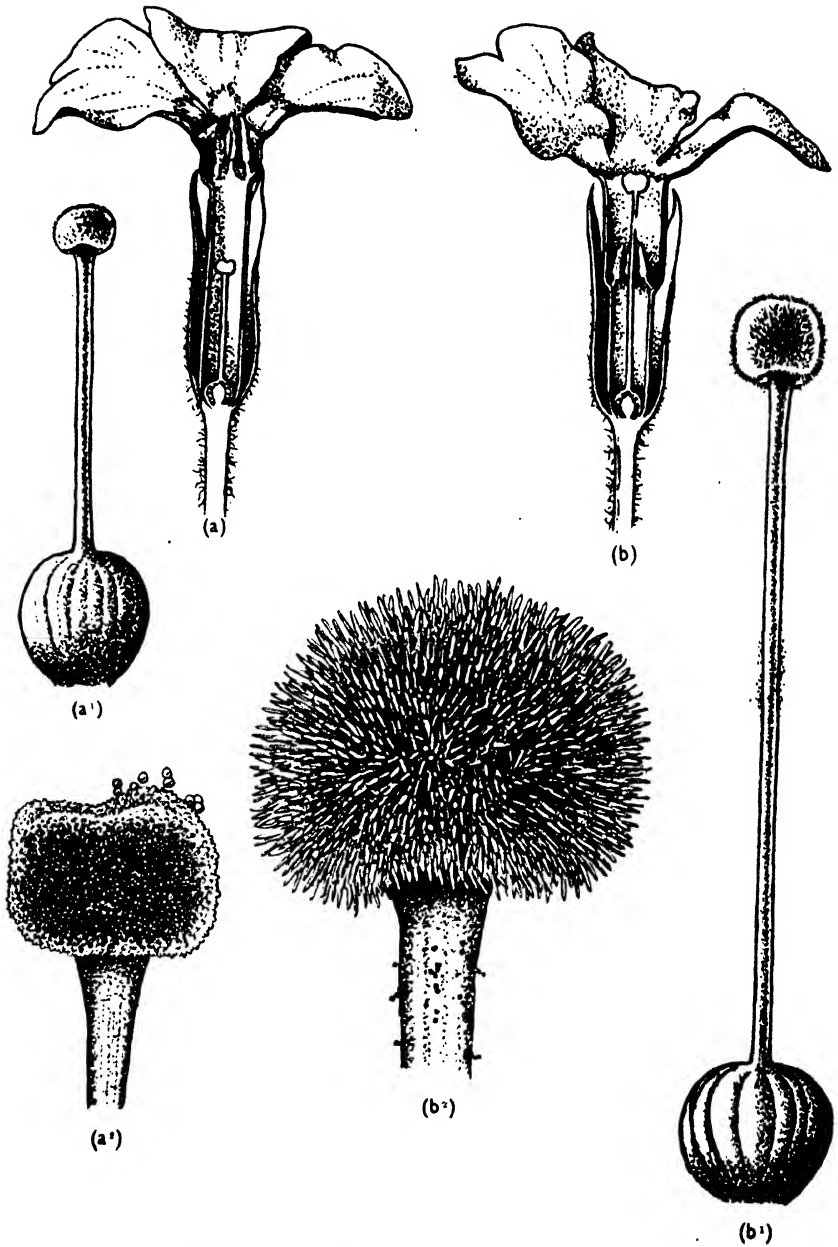


FIG. 163.—Primrose, (a) thrum-eyed, (b) pin-eyed. (a), (b) View of flower in median longitudinal section ($\times 1\frac{1}{2}$), (a'), (b') gynoecium ($\times 5$), (a''), (b'') stigma ($\times 20$).

England the primrose is mainly propagated by seed resulting from self-pollination.

These few cases give some idea of the range of specialisation of flowers to insect visitors. Very few generalisations can be made in this connection, and any species of flowering plant may have some special feature, or combination of features, which renders it particularly suited to specific insect visitors, which in their turn may or may not be of value in bringing about cross-pollination.

Fruit Formation and Seed Dispersal.

When pollination has taken place the primary result is fertilisation and the production of an embryo. This result is associated, however, with so many changes in the floral parts, external to the embryo, that the appearance of the reproductive region entirely alters as fruit replaces flower. Normally the changes are a definite indication of the development of a fertile embryo within and, indeed, may well be regarded as the reaction of the surrounding tissues to the swelling and growth of the embryo, much as the production of a plant gall may be regarded as the reaction of the plant tissues to the development of a larva from an insect egg deposited in the tissues. However, we have to remember that in certain cultivated races of plants, such as certain bananas and oranges, fruit formation occurs although embryo and seed production fails, and thus seedless fruits may be formed.

The ovary wall surrounding the fertile seed naturally takes the most active part in this post-fertilisation development, but other floral parts, including the axis itself, may also develop to form part of the system associated with seed protection and dispersal. There has arisen a convention to describe as "true" fruits those which have arisen from the gynæceum alone, whilst those in which other parts contribute at all prominently are described, somewhat unsuitably, as "spurious." In either case, the remarkable structural developments which follow fertilisation are again most readily interpreted in terms of function. Parts such as petals and stamens, which played a rôle only in pollination, fall or wither to insignificant dimensions, whilst the parts which may have a function in connection with seed protection and dispersal, and therefore particularly the ovary wall, now known as the

pericarp, undergo extensive development ; this is sometimes quite remarkable, as may be realised, for example, if the size of the fruit of an apple or orange is compared with that of the ovary in the flowers of these plants.

Fruits may conveniently be grouped into two large classes. In the one class the carpel contains only one mature seed, in the other several seeds are contained in each carpel or in the syncarpous ovary. In the first class the dispersal of carpels will mean dispersal of individual seeds, whilst in the second the dispersal of separate seeds can only be brought about if the ovary is first burst open by some dehiscence mechanism. Most one-seeded carpels, therefore, give rise to indehiscent fruits, whilst many-seeded carpels or ovaries give rise to dehiscent fruits. An intermediate group includes many types of gynæceum in which the syncarpous ovary contains several one-seeded chambers. Here again dehiscence of the individual compartments is not necessary, but separation of the compartments is provided by some splitting mechanism. Fruits of this kind, schizocarps, are typical of the Umbelliferae, where the carpels of the two-chambered inferior ovary split away from the central part of the septum but remain attached to the stigmas, so that for a time they are present as two pendent, one-seeded portions, which eventually break away (Fig. 164). In the garden geranium (*Pelargonium*), the superior ovary consists of five fused carpels, which are surmounted by a long beak formed from the fused styles. When dry, the one-seeded carpels tear away from the persistent central column and remain for a time suspended from the stigmatic end by a strip of styler tissue. This strip coils spirally on drying, and its subsequent hygroscopic curling and uncurling may play its part in forcing the scattered carpels into the soil. In the mallow (*Malva* spp.) or hollyhock (*Althæa*) the large flat gynæceum breaks up into a large number of light one-seeded segments, and in all members of the family Labiatae, the fruit, formed from two fused carpels, consists of four one-seeded chambers, which ultimately break apart into four small nutlets, which fall separately out of the calyx cup.

Another classification of fruits cuts right across this separation into indehiscent and dehiscent types. From their mode of separation all schizocarps are types which split apart on drying, but both dehiscent and indehiscent fruits may fall

into the categories of either dry or succulent fruits. In succulent types the scattering of the seeds usually occurs after the fleshy covering has been removed. In many-seeded succulent fruits the dehiscence mechanism is usually passive ; the seeds may be liberated simply by the rotting of the suc-

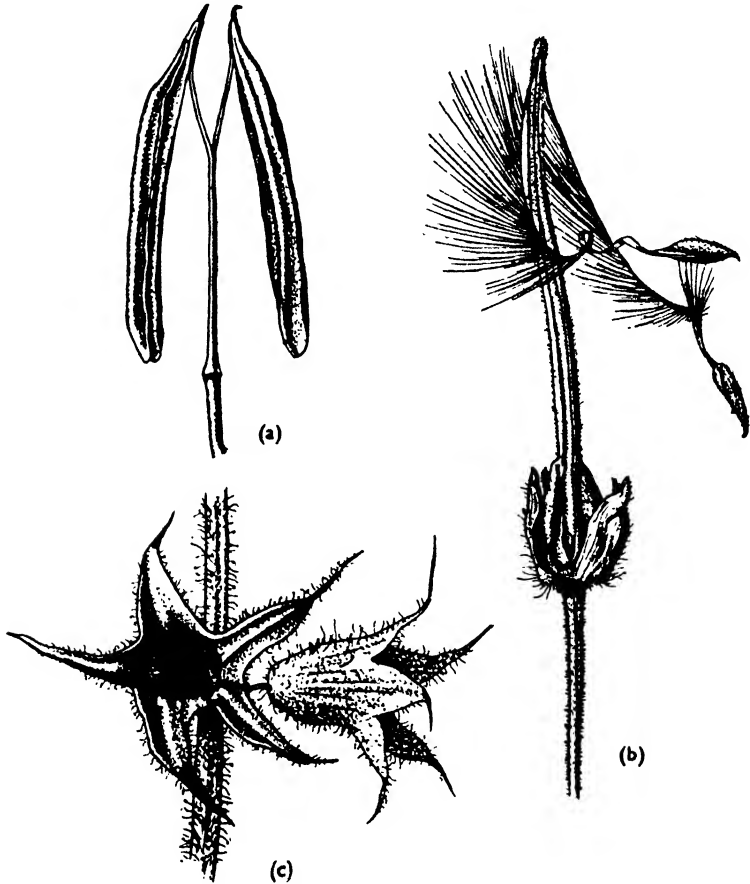


FIG. 164.—Schizocarpic fruits, (a) *Anthriscus* ($\times 6$), (b) *Pelargonium* ($\times 2\frac{1}{2}$), (c) *Lamium* ($\times 3$).

culent part, or more often the seeds become scattered after their passage through the alimentary canal of some bird which feeds upon the pulp. Exceptional cases have to be recorded, such as the squirting cucumber (*Ecballium Elaterium* A. Rich.), where the fruit is swollen with liquid which

eventually reaches such a pressure that the fruit is forced off its stalk, when the liquid contents, including the seeds, are squirted out as the fruit wall contracts.

Indehiscent one-seeded fruits that are dry (achenes), must depend upon other transport mechanisms, which can usually

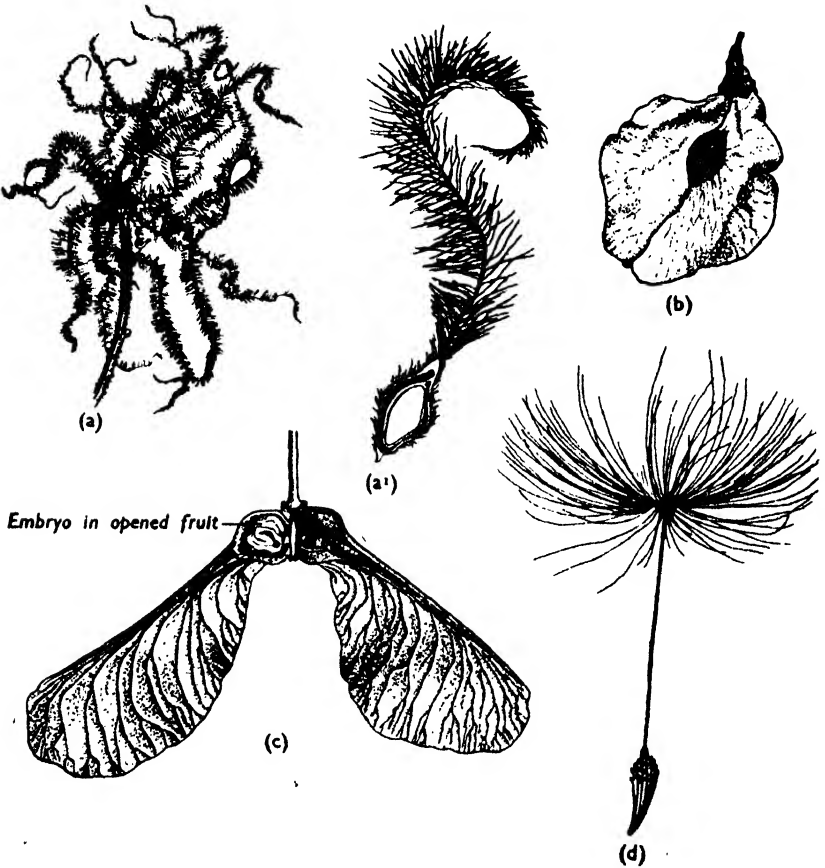


FIG. 165.—(a) Achenes of *Clematis* ($a \times 1$, $a^1 \times .4$), (b) samara of elm ($\times 1\frac{1}{2}$), (c) double samara of sycamore ($\times 1\frac{1}{2}$), (d) cypsela of dandelion ($\times 3\frac{1}{2}$).

be predicted from the structural modifications of the fruit. Thus there are a number of small fruits that have modifications suitable for wind dispersal, such as the achene of the *Clematis* with its long, hairy, persistent style, the samara of ash or elm and the double samara of sycamore in which the pericarp is developed into a wing around the fruit (Fig. 165). The

cypsela of dandelion is formed from the inferior ovary, the upper part of which is surmounted by a beak-like upgrowth bearing a tuft of silky hairs, known as the pappus, and which is regarded as a modified calyx (Fig. 165).

The hooked style of the achene of *Geum* adapts it to cling to the hairy coats of animals moving through the herbage, and similar mechanisms are present in the fruits of *Bidens*, which are covered with downwardly directed barbs, or those of cleavers (*Galium Aparine* L.) with their hooked appendages, and numerous other types.

Indehiscent, one-seeded fruits may be fleshy, when the seed itself is usually enclosed within a hard endocarp or stone, outside which is the fleshy mesocarp, covered by the skin or epicarp; all these three regions are differentiated from the ovary wall and form the pericarp (Fig. 166). Such fruits are known as drupes, the plum and cherry are common examples. In blackberry and in raspberry the gynæceum of the flower consists of a number of separate carpels, closely aggregated on a common receptacle, and when each carpel gives rise to a small drupe or drupel, a collection of drupels arises from the same flower (Fig. 166). Below this the calyx persists and remains of the stamens are often to be recognised. Not far removed from this type of fruit is that of the strawberry, in which the persistent green calyx is crowned by a large succulent, red structure, developed from the receptacle of the flower, upon which the separate carpels have developed into dry achenes (Fig. 166). Since the axis is the predominant part of this compound fruit, it is one which comes under the conventional heading of a "spurious" fruit.

The succulent fruits so far considered, the drupe, the aggregates of drupels, and the spurious fruit of the strawberry, are all examples drawn from the rose family, Rosaceæ, and this family is so varied in the types of fruits developed that still others may be illustrated from amongst its genera. The hip of the rose is a case in which the receptacle is deeply cup-shaped, and it is this which becomes coloured and fleshy, and which surrounds the true fruits, which are dry achenes with long, hairy styles which protrude through the mouth of the hollow receptacle, where also the persistent segments of the calyx are present (Fig. 167). In the haw of the hawthorn (*Crataegus monogyna* Jacq.), the receptacle is again deeply

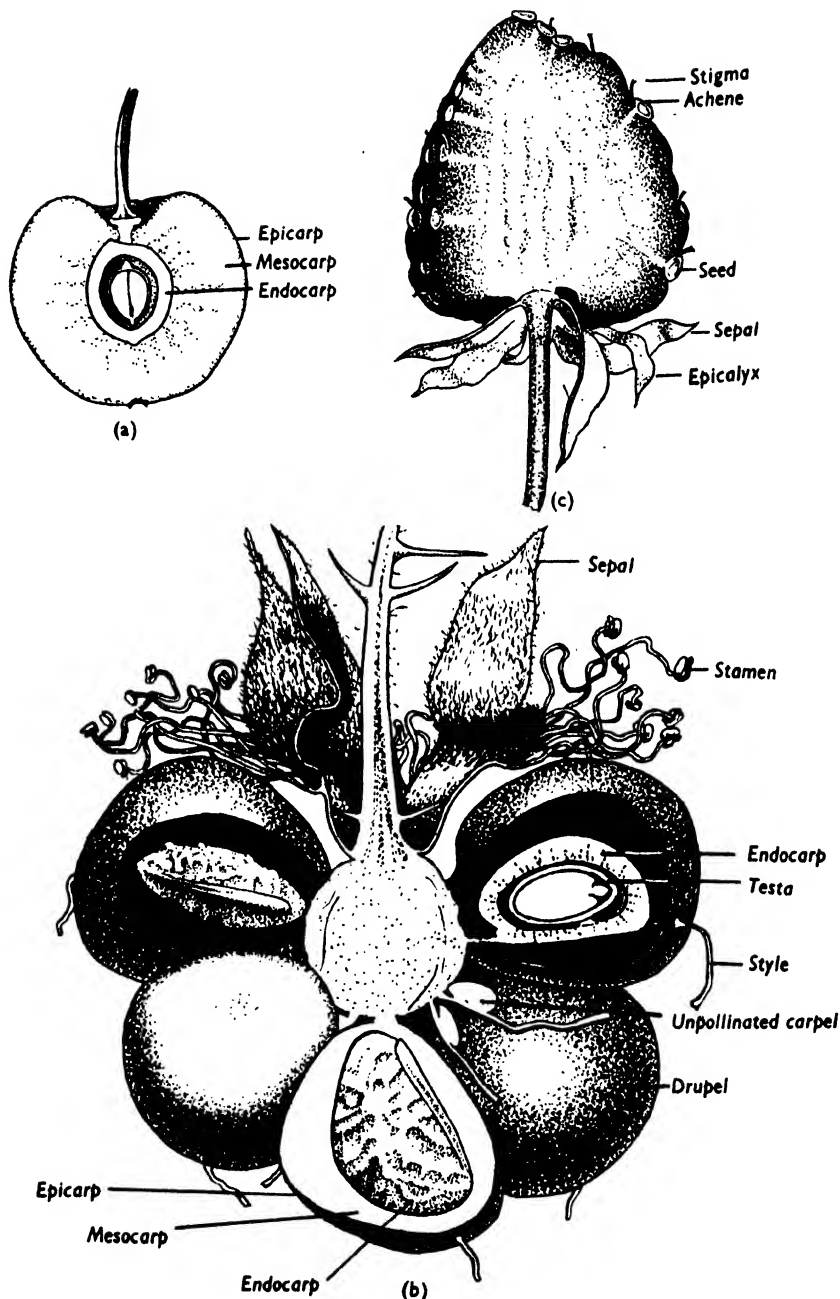


FIG. 166.—Fruits of (a) cherry ($\times 1\frac{1}{2}$), (b) blackberry ($\times 7\frac{1}{2}$), (c) strawberry ($\times 1\frac{1}{2}$), in median longitudinal view.

cup-shaped, and almost fused with the stony pericarp; in this species the carpel is usually single and functions as an achene, as the second ovule does not normally develop into a

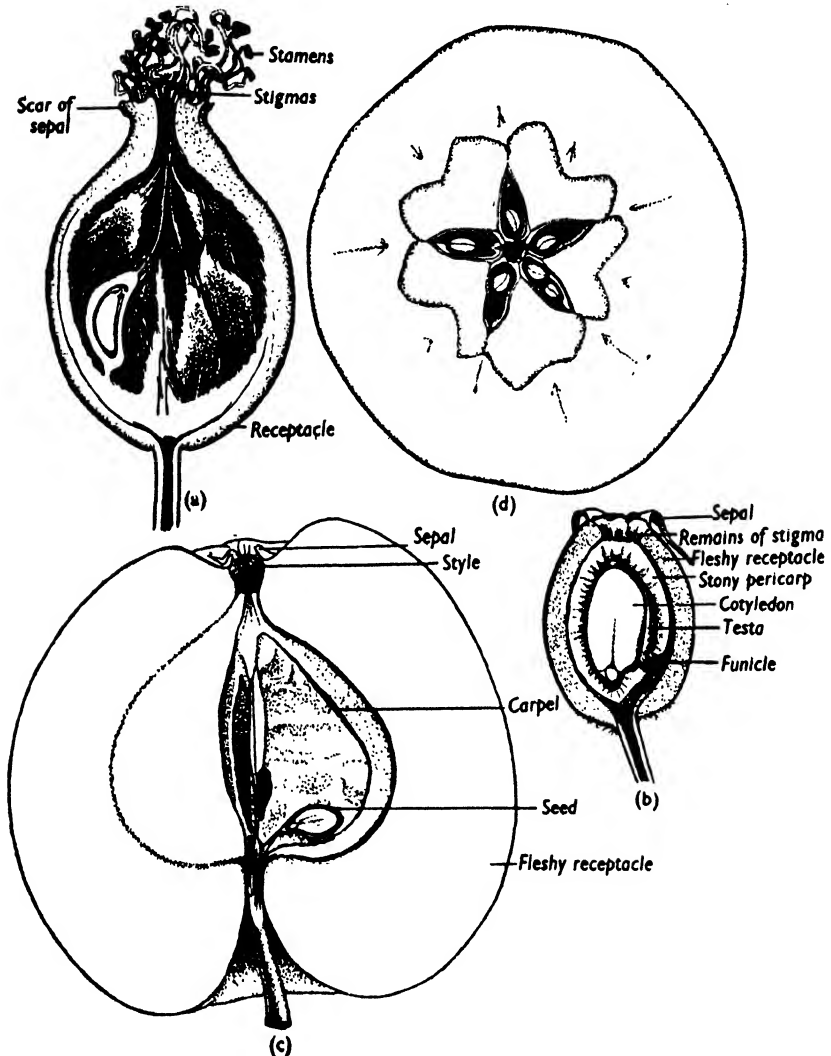


FIG. 167.—Fruit of (a) rose ($\times 2$), (b) hawthorn ($\times 3\frac{1}{2}$), (c) apple in median longitudinal view, and (d) apple in transverse view ($\times 1$).

fertile seed (Fig. 167). In the apple, the receptacle is deeply cup-shaped and fits closely around the five carpels, which are several-seeded. As the fruit, a pome, develops, the carpels

become more or less fused with one another and with the receptacle. The seeds are enclosed in the core, which is formed by the horny development of the inner layers of the pericarp; this is enclosed in the fleshy part of the fruit, developed partly from the pericarp and mainly from the receptacle (Fig. 167).

The examples considered so far have been a few typical examples of indehiscent fruits, either dry or succulent; in addition to these we must also illustrate the range of structures shown by many-seeded fruits. Amongst dry, dehiscent fruits of this class, which are included under the general term of capsule, attention has been concentrated mainly on the method of dehiscence. The commonest method is perhaps a lengthwise splitting, when the wall separates into valves; the splitting may take place along the median plane of a loculus, when it is known as loculicidal, e.g. *Iris* (Fig. 168a), or it may take place along the length of the septum, when it is known as septicidal, e.g. many species of St. John's wort (*Hypericum* spp.). In a multilocular ovary the septa may also be broken across so that a central column of the original placenta is left erect in the centre of the dehiscent fruit and isolated from the valves, a condition known as septifragal, thus in *Enothera* the dehiscence is loculicidal and septifragal, in *Rhododendron* septicidal and septifragal. In some capsules, opening by longitudinal splitting, the rupture does not extend far down the ovary wall and merely results in the formation of a number of teeth, as in the bladder campion (*Silene inflata* Sm.), in which the ovary is unilocular with free central placentation.

On the other hand, the dehiscence may be more localised, as in the porous dehiscence of *Antirrhinum* (Fig. 168b) and poppy (*Papaver* spp.). In these two cases the pores are at the distal ends of erect fruits, so that the tiny seeds are shaken out through pores when the wind is strong enough to shake the fruit stalks vigorously, an arrangement spoken of sometimes as a censer mechanism. It is certainly suggestive to compare with these the capsules of *Campanula* (Fig. 168c), formed from inferior ovaries, which are inverted when ripe, and in which the pores are found at the proximal ends of each loculus, so that again the seeds will not be shaken out unless the plant is well shaken by the wind. In *Plantago* the capsule, or pyxis, dehisces by the separation of a circular lid.

In many of these types, in addition to the dehiscence mechanism of the capsule, the seeds may also have some structural feature which will assist in their dispersal. In

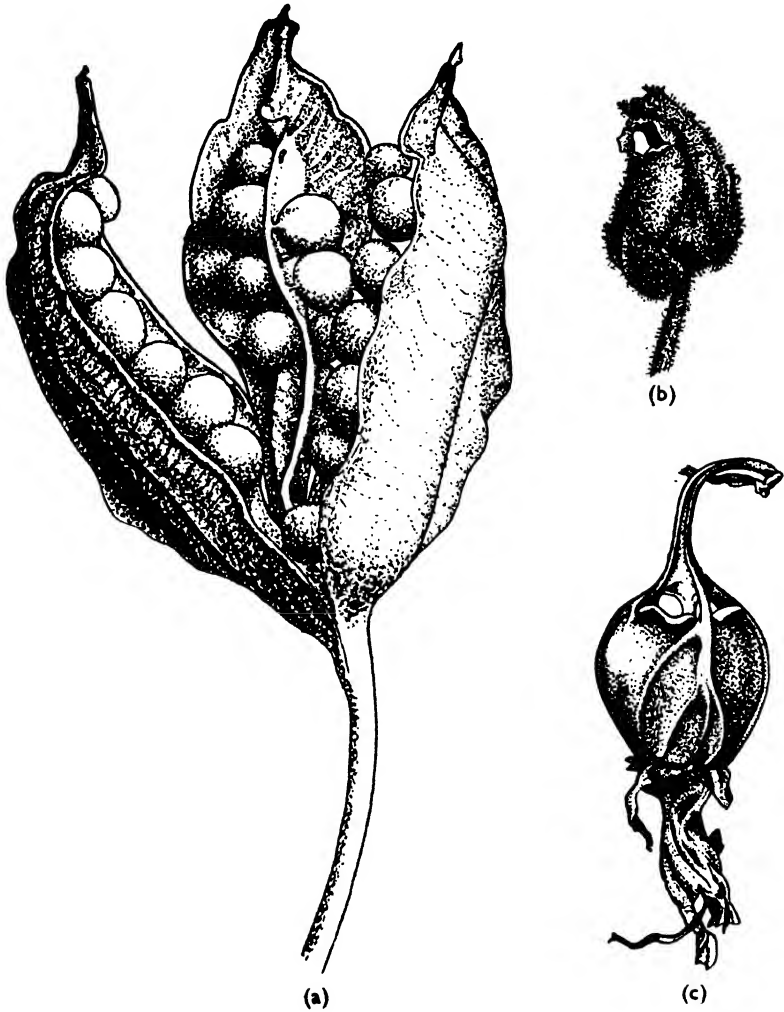


FIG. 168.—Dehiscence of capsules, valvular (loculicidal) in (a) *Iris* ($\times 2$), porous dehiscence in (b) *Antirrhinum* and (c) *Campanula* ($\times 2$).

yellow rattle (*Rhinanthus*) the seeds have the testa developed into a marginal wing, whilst the seeds of willow, poplar, and willow herb (*Epilobium*) have an outgrowth of hairs.

The separate carpels of many genera of the Ranunculaceæ are many-seeded and dehiscent. Whilst the genus *Ranunculus* itself has a fruit consisting of small, one-seeded, indehiscent achenes, with little indication of structural features to facilitate

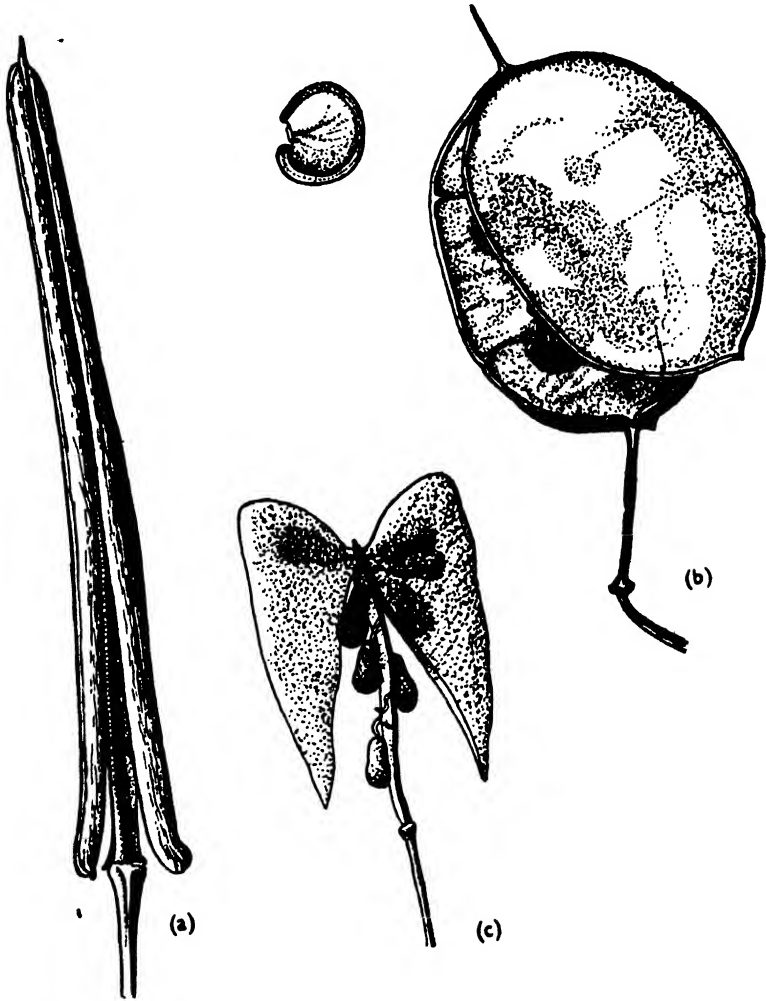


FIG. 169.—(a) Siliqua of stock ($\times 2$), (b) silicula and seed of *Lunaria* ($\times 1\frac{1}{2}$), (c) silicula of *Capsella* ($\times 6$).

dispersal, other than a slightly hooked style, in the genera *Caltha*, *Delphinium*, *Aconitum*, etc., the separate many-seeded carpels split open along the ventral suture only. Such dehiscent carpels are described as follicles and are distinguished

from the legumes, the single carpels of the pea family, Leguminosæ, which dehisce along both sutures, often with explosive violence and a spiral twisting of the carpel valves.

Equally well known are the characteristic, two-chambered, dehiscent fruits of the wallflower family (Cruciferæ). These fruits have parietal placentation, but a partition, known as the replum, runs across the ovary. When the fruits dry on ripening, the two valves split away from the bottom, leaving the replum still erect in the centre. The force with which the valves separate usually scatters the ripe seeds, though some may remain for a time attached to the replum. These crucifer fruits fall into two general categories, the siliqua, in which the fruit is very elongated as compared with its breadth (Fig. 166), and the silicula, in which the fruit is short and flattened, the flattening sometimes being in the plane of the replum, as in honesty (*Lunaria*), sometimes at right angles to it, as in shepherd's purse (*Capsella Bursa-pastoris* Med.) (Fig. 169).

There are also a number of succulent, many-seeded fruits, in which there are present one or more loculi, according to whether the manner of placentation by which the seeds are arranged is axile (tomato, orange), or parietal (gooseberry). In tomato (*Solanum Lycopersicum* L.) the position of the calyx, near the fruit stalk, shows that the fruit is developed from a superior ovary, in gooseberry the persistent remains of the corolla at the distal end shows that it arises from an inferior ovary. In all these berries the testa itself forms the hard protective layer to the seed, whilst the pericarp is entirely fleshy. In this respect berries differ from drupes or pomes, in which the hard layer, protecting relatively thin-coated seeds, was formed from the inner part of the pericarp.

CHAPTER XXXIX.

PLANT CLASSIFICATION.

PLANTS were first studied so that one kind might be distinguished from another. They are now studied for their own intrinsic interest, but the naming and grouping of plants still remains essential to their study, and we may now review such knowledge as we have gained to see how it may be applied to the process of plant classification.

It was necessary at the outset to indicate some of the main groups of plants so that the immediate object of study, the flowering plant, could be more closely defined. The examination of this one type of plant alone, however, has revealed an almost infinite diversity of form and structure within the group. At the same time we have learnt that the different kinds are faithfully reproduced, even if with a characteristic range of variation of form and structure within each species. It has been one of the aims of the botanist, particularly the taxonomist, to name and describe each of the many species in such a way that anyone may be able to recognise it again, so that not only will the plant be known and named, but with it will gradually become associated various properties and a certain geographical distribution. As the binomial names given to each species are Latin, and common to botanists of all countries, this ideal of a knowledge of the properties and distribution of all plant species becomes more complete each year, the facts being embodied in the floras that are published by the taxonomists of different countries. This information is now particularly complete about the flowering plants, and it is a very rare occurrence in a long-inhabited country, like the British Isles, for a new species of flowering plant to be added to the flora, though

new species of algæ or fungi are not infrequently described. On the other hand, in a country relatively recently populated by a civilised race, intensive study of the flowering plants would still reveal many unidentified or incompletely described species.

When a botanist wishes to name a plant, his first task is to look through previous lists and descriptions of species, to see if this plant is amongst them. This task will be facilitated if the previously described plants are arranged in some orderly manner, so that he may expect to find his plant, if already described, in a particular part of the long list. This need for system has long been in the minds of taxonomists, and the founder of modern taxonomy, the great botanist, Linnæus, first arranged plants according to an artificial system. He grouped them primarily according to the number of carpels present in the flower, and then subdivided these groups on a basis of stamen number. Though there is always a danger that a species may vary in respect of one isolated character of this kind, if it is thus used too exclusively as the basis of grouping, yet this artificial system proved of great value in facilitating the search for a plant amongst those already named and described.

However, Linnæus himself recognised that a grouping of plants based in the first place solely upon one such particular character often did violence to the true affinities of the plants considered ; by such a system certain plants, which differed widely in many other respects, came to be closely grouped, and in other cases plants with many other characters in common came to be widely separated.

But what do we mean by the suggestion that plants could be regarded as close together or far apart in their affinities ? It is a concept that nowadays comes very naturally to the mind of any student of taxonomy. He cannot resist the impression that two species, although quite distinct in certain characters, are so alike in many others that their affinity must be close, whilst others differ in so many respects that their affinity should be regarded as remote. This impression has had definite significance since the publication of "The Origin of Species," by Charles Darwin, and the acceptance by the biologist of the idea of a process of evolution. Since the publication of this book, the continued accumulation of

evidence has probably convinced all biologists that the groups we call species, though well defined and constant for all practical purposes during our life-time, and therefore capable of definition, are yet themselves subject to change in the course of time. We now realise that plants have existed on the surface of the globe for a very long time, to be measured in terms of millions of years, and that during this time they have undergone change. Some species have died out and been replaced by others that have been derived from them. Thus a new significance is given to the instinctive conclusion of the taxonomist: two species which seem closely allied may be regarded as derived comparatively recently from a common ancestral species, which may be still represented or may have become extinct. On the other hand, two species which seem only remotely related may have diverged from a common ancestor much further back, if indeed they ever had a common ancestor, a point that obviously remains purely speculative, though its assumption makes much more intelligible the many points in common that are possessed by even widely divergent types of vegetable life.

This evolutionary standpoint introduces a new idea into the classification of plants. We may try to arrange them in such a way that, whilst still having every facility to ascertain whether a plant has previously been named and described, the grouping may yet do as little violence as possible to what seem to be the natural affinities of the plants, such a grouping may be regarded as an expression of their degree of closeness or remoteness of evolutionary descent.

Such a grouping cannot rely upon any single character. There is no one character that we can regard as more stable than another with the passage of time, nor can we contemplate that change has taken place always to the same degree, in the same plant organs, along different lines of descent. The grouping must always depend upon a delicate balance of judgment as to the relative significance of the sum of all the characters studied, and it is natural, therefore, that such a natural classification of plants should be liable to modification with every addition to botanical knowledge. Alterations do not mean, of course, that the evolutionary history of the plants alters, but merely that our views of that history, which will

always remain speculative and incomplete, become modified with increased information, and we may hope are becoming more accurate.

It is clear, however, that a natural classification of plants is but an ideal towards which we can hope to make progress but which we can never hope to attain, and there is little wonder that our ideas as to these groups, the species, genera, and families into which we arrange the known forms of plants, are only tentative, and often there is no complete agreement amongst botanists as to the range of form in certain types which should be included in one species.

Nevertheless, species, genera and families are fairly definite concepts, and it is important that any student of plants should familiarise himself with them in relation to examples taken from the local flora, and should make a comparison of the distinctions that are used to separate species from species, genus from genus, and family from family. Such work can only be done by examination of the actual specimens, and the student is recommended at this stage to attempt two types of practical exercise which will lead to a much greater familiarity with the plants of the neighbourhood in which he lives. No other exercises will give him a better acquaintance with plants and none will more quickly make him conversant with that range of diversity in plant form, which is at first so bewildering to the beginner and afterwards so fascinating an accompaniment to their study.

In the first place, he should attempt to use one of the many keys, or floras, for the naming of plants, so that he can himself identify them. At first he will need to refer to some more experienced person to confirm the accuracy of his naming. Later he will become more confident in the use of the flora, and if in doubt will be able to check his identification either by reference to descriptions and figures of the species, or, better still, by comparison of his plant with an authentically named specimen in a herbarium collection. If such taxonomic work appeals to him, he will soon have the wish to make his own named collection of dried herbarium specimens for reference. Any competent naturalist will advise him as to the preserving and mounting of his own herbarium specimens, or reference may be made to such a book as "Descriptive Systematic Botany," by Hitchcock.

The beginner will find the "Illustrated Guide to the Trees and Flowers of England and Wales," by H. G. Jameson, a very useful book for his first efforts in naming plants for himself. This book does not attempt to distinguish the species of grasses and sedges, but with growing experience the student will find himself able to use such a standard flora as Bentham and Hooker, or the convenient pocket flora, "Hayward's Botanists' Pocket Book," revised by Druce.

The other type of exercise that is necessary to extend the beginner's experience of the significance of a natural classification is the more intensive study of a few species and genera, and natural families.

If the student's studies are following the University terms, he is probably ready for this study in the early summer months, when as yet relatively few flowers are fully out. At this time he cannot do better than take a few species of the genus *Ranunculus* for close comparison with one another, and then this genus may be compared with other genera of the same family, when he will be in a position to estimate the kind of differences that are used to distinguish species, and those used to distinguish genera. He may then pass on to consider what characters these genera have in common which have led to their grouping together in the family Ranunculaceæ, as compared with other families such as the Cruciferæ and Rosaceæ.

One general point will then at once emerge. It was pointed out in the last chapter that the characters of the reproductive parts of a plant, flower, and fruit, though differing from species to species, show relatively little variation within the species. This conservatism of reproductive structures seems to have been true over long epochs of time, and the result is that, whilst vegetative characters may be used in building up the balance of differences which distinguish species from species, where we have to find the more fundamental differences, which distinguish genus from genus, and family from family, we rely more and more upon characters associated with reproduction.

Another point to notice particularly is that no specific or other distinction rests upon single characters alone. Distinctions always rest upon a balanced judgment of the significance of a number of differences, and, if the judgment is

a good one, increasing knowledge of the range of form, the details of structure, and even the physiology of the plants, will only confirm the conclusion reached.

In the buttercup or crowfoot genus, *Ranunculus*, the species fall into two groups, white-flowered, aquatic species and yellow-flowered, terrestrial species. Amongst the white-flowered species we may distinguish *Ranunculus fluitans* Lam., as this plant grows in running water, all the leaves are submerged and finely divided into thread-like segments surrounding the veins; the carpels and the receptacle are quite smooth and free from hairs. *Ranunculus peltatus* Schrank, grows in ponds. In addition to sub-divided, submerged leaves, it usually bears lobed, floating leaves, in the absence of the latter, however, it may still be distinguished from *R. fluitans* by the presence of hairs on the carpels and receptacle. A third species, *R. hederaceus* L., grows on mud and has leaves of the lobed type only; like *R. fluitans*, the carpels and receptacle are glabrous, but the flowers are large in *R. fluitans*, small in *R. hederaceus*. In the latter species there is also less difference in the length of sepals and petals.

Amongst yellow-flowered species, *R. Ficaria* L. may be distinguished by flower characters, the numerous petals (eight to twelve) together with a few yellowish sepals (three). Other useful distinguishing characters of this species are the heart-shaped leaves and the swollen roots. Similar root tubers very readily arise also from the buds in the axils of the leaves in some forms. The majority of the yellow-flowered species have five sepals and five petals. *R. Flammula* L. is a marsh species known from most others by its narrow and entire or slightly toothed leaves, which have given it its name of lesser spearwort. *R. Lingua* L., the great spearwort, is similar in general form but is a much larger plant, which also differs slightly in the form of the carpels. The other three species which we will consider, *R. bulbosus* L., *R. repens* L., and *R. acris* L., all have deeply divided, palmately veined leaves. *R. bulbosus*, as its specific name suggests, is characterised by a swelling of the base of the stem at ground level, whilst another useful character is the reflexing of the sepals against the ribbed flower stalk.

R. repens and *R. acris* are easily distinguished from *R. bulbosus* as they have neither the reflexed sepals nor the swollen

stem of the latter, but they are slightly more difficult to tell from one another. In well-grown plants the habit is characteristic, for whilst *R. acris* is an erect plant with a branched flowering axis, *R. repens* has the characteristic stoloniferous habit, described earlier in this book. The leaves of both species are hairy, but those of *R. acris* tend to be rather more finely divided and the middle lobe is less marked than in *R. repens*. When they are found growing together it is noticeable that the flowers of *R. acris* are smaller and paler than the larger and deeper yellow ones of *R. repens*, and also the flower stalks of *R. repens* are furrowed whilst those of *R. acris* are smooth.

Whilst floral characters have been used in making the specific distinctions, practically equal significance has been attached to various vegetative characters. If a similar analysis is made of the grounds of separation of the genus *Ranunculus* from other genera of the Ranunculaceæ, it will be found that relatively more significance is attached to reproductive structures, and notably the characters of the carpels and resulting fruits. Those with a single ovule in each carpel give rise to achenes, the types with carpels containing many ovules give rise to such fruits as the follicles of *Caltha*, *Delphinium*, *Aquilegia*, etc., the capsule of *Nigella* or the berry of *Actæa*.

Achenes occur in *Ranunculus*, *Clematis* and *Anemone*, but in the two latter genera true petals are lacking, and the calyx is coloured; in *Anemone* also a characteristic whorl of bracts occurs beneath the flower. The achenes of *Clematis* are usually distinguished by the hairy development of the style, whilst *Ranunculus* and *Anemone* have the point of difference that in *Ranunculus* the single ovule in the carpel is attached to the floor of the carpel cavity (basal), and in *Anemone* it is suspended from the stigmatic end. The distinctions between these two latter genera are, however, so fine that it has been possible for a New Zealand plant to pass for many years as a *Ranunculus*, which quite recently has been shown actually to be an *Anemone*, the only representative of this genus in the New Zealand flora. The petaloid sepals of *Caltha* and *Helleborus* are also diagnostic characters, together with the complete absence of petals in *Caltha*, and their replacement in *Helleborus* by the remarkable slipper-shaped nectaries, which seem to be comparable with the nectaries present at the base of the petals in all species of *Ranunculus*, where in many species they are covered by a

small scale. Similarly, the genus *Trollius* is distinguished by the presence of numerous wide, yellow sepals, which enclose the narrow, dark yellow petals and the remaining parts of the flower, the genus *Aquilegia* by the five spurred petals, *Delphinium* and *Aconitum* by their zygomorphic symmetry and other peculiarities of construction. Characteristics of form and habitat are often associated with other generic distinctions, such as the rounded leaves and hollow stem of the marsh plant *Caltha*, the large, palmately divided leaves of *Helleborus*, the finely divided leaves of *Thalictrum*, *Actæa*, and *Nigella*, the climbing habit of *Clematis* which is diagnostic, but in most cases the generic differences are better based on reproductive characters.

When the family Ranunculaceæ is contrasted with its neighbours in a natural classification, the most important distinguishing characters are again found to be floral. As there is such a comparatively wide range of floral characters in the family Ranunculaceæ, those common to the group are not readily recognised at first. In that respect it may be well to contrast the Ranunculaceæ with a family like the Cruciferæ, in which the characters of both flower and fruit are exceptionally constant, and with the Rosaceæ, the family with floral characteristics which the beginner most tends to confuse with the Ranunculaceæ, and which is also a family which illustrates well the wide diversity of form which may be grouped in one family.

The floral characters which are common to the genera of the Ranunculaceæ are associated particularly with stamens and carpels. As we have seen, the members of the calyx and corolla vary considerably in number and form; petals may be absent altogether or modified into nectaries, when the sepals usually become petaloid or coloured. In the species of *Ranunculus* passed in review, the petal number in *R. Ficaria* was relatively high, usually about eight or nine, but in the majority of the species the number was five. This definite number suggests a whorl of petals, inserted at the same level on the flower axis, but on the other hand both stamens and carpels in *Ranunculus* are not only numerous, but indefinite in number, and though in other genera the number of carpels falls, even to one in *Actæa*, it is usually rather variable in any species, and the number of stamens is always indefinite. The indefinite number is the natural consequence of the way in

which the stamens are arranged, and is a point for comparison with the Rosaceæ, where the stamens are also often numerous but usually in multiples of five, because arranged in whorls of which the basic number is five. In the Ranunculaceæ they are clearly arranged in a spiral manner around the elongated axis of the flower, though the absence of internodes between successive insertions makes the spiral a very close one ; it is possible to see, however, that the outer stamens are inserted lower on the axis and are older, and within them progressively younger stamens, and then carpels, are arranged in a spiral succession upon the conical axis, so that the carpels are inserted not only nearer the centre of the flower, but a little higher on its axis than the stamens. The flower is therefore hypogynous, another important character of the family. The spiral seems to run smoothly on from insertion of stamens to insertion of carpels, and study of a number of flowers will certainly show structures transitional between one category of floral member and another.

Since spiral phyllotaxis is so constant a feature of the vegetative axis, and the floral parts follow the vegetative on the same axis, there is a natural tendency to regard the spiral arrangement of floral parts as primitive, and to consider that this arrangement has been replaced in the course of time by a tendency for the parts to emerge in whorls. The whorled arrangement is usually associated with a shorter floral axis so that the insertions of the floral parts are more crowded.

Another character which is common to all the Ranunculaceæ, except in some degree to *Nigella* and to a few other genera, is the complete freedom of the individual members of all whorls from one another, the sepals are polysepalous, the petals polypetalous, the stamens free and, still more important in the characterisation of the family, the gynæceum is apocarpous. Two genera give some difficulty in this respect—*Nigella*, where the carpels show a definite tendency to fusion giving a fruit which is a capsule, and *Actæa*, where only a single carpel is present in the flower, and this gives a fruit which is a berry, the fruits of other genera being dry. However, the other characters of these genera leave little doubt that they are correctly placed in this family.

It will be seen that the floral characters that can be

associated particularly with this family, though important, are relatively few. It is not possible to add vegetative characters. The spiral arrangement of leaves, which are exstipulate, is also common to the plants of too many other families, and in any case *Clematis*, with decussate leaves, is an exception in this respect. Again, if it were not for the fact that *Clematis* is a woody climber, we might have added that all the members of this family are herbaceous, but though the habit of the plants in some families may be very constant, yet in others it may be extremely varied, and it is seldom a point which can very usefully be employed in the effort to assign a plant to a family.

We may now contrast with the relative range of variability in floral structure in the Ranunculaceæ, the floral uniformity that is found in a family containing a far larger number of genera, the wallflower family, Cruciferæ. Here all the plants are herbaceous, with leaves inserted singly according to some spiral system, and the whole vegetative habit is comparatively uniform, but the plants could not be recognised with any certainty as belonging to this family by vegetative characters alone, whilst even the beginner, after study of the flower of wallflower and

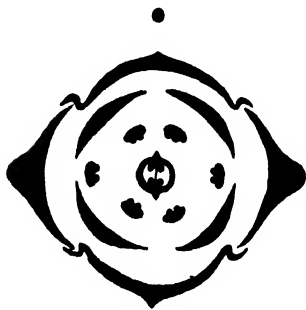


FIG. 170.—Floral diagram of wallflower.

one or two other genera, would subsequently recognise plants of this family without difficulty.

In this case the facts may be very simply stated and illustrated in the form of a floral diagram and floral formula. The diagram shown in Fig. 170 is actually of the wallflower, but in almost every detail it would hold for almost any genus of the family. In most of the Cruciferæ the bracts subtending the flowers fail to develop. The sepals will be seen to lie in two whorls of two, at right angles to one another, the members of the lateral pair being a little pouched at the base (gibbous), thus giving a cavity in which nectar collects from the nectaries at the base of the lateral stamens. Actually, in order of origin, the lateral pair of sepals appear to be the outer whorl, though from the way in which the sepals overlap in the adult flower the median ones appear to be the outer. These two

whorls of sepals are represented in the floral formula as $K_2 + 2$. The petals are in one whorl and polypetalous, and from the diagram it will be seen that the petals appear opposite the gaps between the sepals. The individual petals are also very characteristic, with a long narrow claw tapering to the insertion and the distal end expanded into a wide spreading limb. The construction of floral diagrams for different families soon makes it clear how frequently the members of successive whorls arise in the gaps between those of the whorl external to them, a phenomenon which is readily understood when the growth of the flower is considered, and we realise how crowded the primordia of the floral parts must be at their first origin, and consequently how much the position of their emergence must be influenced by that of the primordia which just preceded them. When such alternation of members of successive whorls does not occur, it is a feature that is particularly noted and is often useful in characterising a family: an example is found in the Primulaceæ, in which the epipetalous stamens arise opposite to the petals.

In the Cruciferæ the androecium is very characteristic, and consists of an outer whorl of two lateral stamens with shorter filaments, and an inner whorl of two pairs of stamens with longer filaments (tetradynamous), indicated in the formula $A_2 + 2^2$. The dehiscence of the anthers is introrse. The gynæceum is also very characteristic and consists of two fused carpels with parietal placentation, and with an anomalous partition, the replum, across the cavity. Another curious feature of this gynæceum is the position of the stigmatic lobes. Usually lines of separation between the stigmatic lobes coincide with the planes of fusion of the carpels, so that we should expect the stigma lobes to lie in the lateral plane of the flower, but actually the two lobes lie in the antero-posterior plane, which is also the plane of the replum.

Thus the Cruciferæ are seen to possess a most characteristic and uniform flower structure represented by the floral formula $K_2 + 2, C_4, A_2 + 2^2, \underline{G(2)}$. The hypogynous flower, the tetradynamous stamens, and the peculiar gynæceum are particularly valuable in identification, though the calyx of $2 + 2$ sepals, with one pair gibbous, and the cruciate corolla are also characteristic and useful features. As was pointed out in the previous chapter, the gynæceum also gives rise to

a characteristic fruit, the siliqua or silicula, which always dehisces by valves breaking away from the base. One interesting exception to this may be noted in the fruit of *Isatis tinctoria* L., a native plant from which the ancient Britons extracted the blue dye, woad. This plant is nearly extinct now as a wild plant, though until recently it was still cultivated in Lincolnshire for use in dyeing the blue cloth of the policeman's uniform. This plant has the typical flower and gynæceum, but gives rise to a flattened fruit, not unlike a siliqua in appearance, but which contains only one seed, and therefore behaves as an indehiscent, winged fruit.

There can be no difficulty in distinguishing between the Ranunculaceæ and the Cruciferæ, but there is more likelihood of some confusion between the Rosaceæ and the Ranunculaceæ. The family Rosaceæ is large, and the plants grouped in it show a wide range of habit, varying from herbs to shrubs and trees.

The number of stamens may be small (four in *Alchemilla*) when little confusion with Ranunculaceæ is likely to arise, but in the majority of the genera they are numerous (as in strawberry, cherry or rose), and then more care is necessary in distinguishing the two families. Careful examination shows that in the Rosaceæ the stamens, though numerous, are present usually in multiples of five, and are arranged in whorls, with no suggestion of the indefinite spirals so characteristic of the Ranunculaceæ. A vegetative character which is also helpful to the beginner, though it is dangerous to place too much reliance upon it, is the presence of a pair of stipules at the base of each leaf. The more fundamental floral difference, associated with the relative position of the floral parts upon the axis, is often more difficult to elucidate, but may be recognised if a flower of a buttercup is carefully compared with that of a strawberry (*Fragaria vesca* L.), in median longitudinal section. In the strawberry, the sepals are slightly fused at the base, and are continuous with a saucer-like rim on which the petals and stamens are inserted, whilst from the centre arises a conical upgrowth bearing the carpels only. In many of the genera the degree of perigyny is more marked and easier to recognise than in *Fragaria*, the receptacle may take the form of a shallow cup which bears the calyx, corolla, and andrœcium marginally, as in plum and cherry (*Prunus*),

when the gynæceum is borne on the floor of the cup, or the receptacle may become a relatively deep cup with the carpels arising from the base, as in *Rosa* or, in more extreme cases, the hollow receptacle may so closely invest the carpels as to become fused with the pericarp, as in pear and apple (*Malus*) and hawthorn (*Cratægus*), a feature already considered in the last chapter. The great variety of fruit types in this family are to be traced mainly to this range in the form of the receptacle or calyx cup, through all degrees of perigyny to epigyny.

The range of fruit structure in this family has already been drawn upon in the last chapter to illustrate the different kinds of fruit ; it is actually made larger still by the wide range in the number of carpels composing the gynæceum. In *Fragaria* they are very numerous, and are seen as the dry achenes raised on the fleshy receptacle ; around this the calyx cup persists as a green structure, and the additional feature may be noticed that this appears to be double, with a second series of scales outside and alternating with the sepals, the outer series forming the epicalyx. Carpels are also numerous in *Rubus* and *Rosa*, five in *Malus*, two in the burr-like fruits of *Agrimonia*, and one in the genus *Prunus*. In the latter the cup-like receptacle is clearly seen in the flower, but during fruit development, the greater part of this, with the calyx, corolla and remains of stamens, is shed, leaving only a small collar at the base of the fruit where it joins the fruit stalk.

In this family, then, we have to reconcile ourselves to a very wide diversity of fruit structures ; this, however, is derived from a common floral type, characterised by a whorled arrangement of stamens, though these vary in number from numerous in most genera to only one whorl, or, in one species of *Alchemilla*, only one stamen. Some experience is necessary to recognise the flowers which belong to this family, and in the case of some genera, such as *Fragaria*, with numerous free carpels, there is liable to be some confusion at first with members of the Ranunculacææ. This is a case where the beginner will first find himself attaching importance to the features of stipulate as against exstipulate leaves, cyclic as against spiral insertion of stamens, etc., and only with growing experience will he find himself increasingly alive to the significance of the differences in insertion of floral parts, conveyed in the contrasting terms of hypogyny and perigyny.

This very brief discussion of the characteristics of three common families of the British flora must suffice as an indication of the varying bases of comparison upon which such a group as a family is founded, as compared with a genus or a species. Whether these or other examples are first studied, it will probably be profitable to make a beginning in floral studies along the two lines suggested, on the one hand to learn to identify any flowering plant met with in the field by the use of a suitable flora, and on the other hand to gain familiarity with the concepts of species, genus and family by the intensive study of certain groups of species and genera from one or two selected families. The reader will then find that, as new plants are identified, the characteristics of the new genus and of its family begin to have more significance and, almost before he is aware of it, he will have met with so many examples of species of common British families that their general characteristics will have become familiar to him, even without very specific study of the families themselves. In any case, this last aspect of plant study is a fit and proper place to leave our preliminary survey of the plant, and nowhere can it be more clear that the only method of continuous progress in this field of work is through continued observation and examination of the plant material itself. By the time that the beginner has made the acquaintance of a number of species from the local flora and familiarised himself with the general principles that underlie a natural classification, he will know whether this study has the appeal for him which it makes to the true taxonomist, who is a man born to his task and not made in the study. If this is not his *métier*, yet he may have gained sufficient interest in some branch of botanical study to persist in the tasks, both of naming plants and of familiarising himself with the aims and methods of a natural system of classification. This is in any case to be desired because, whatever the branch of botanical study which is attempted, it will always be more soundly based if the investigator can approach it from a wide experience of the range of structure and degree of variability that is characteristic of the material forming the basis of his study.

INDEX

- ABSCISSION**, 118, 123, 141, 365, 563.
 absorbing zone, 389, 405, 410, 421.
 accessory, bud, 65, 151.
 — cell, 370-1.
 accumulation of salts, 420-1.
Acer Pseudoplatanus, bud scale, 147.
 — fruit, 586, Fig. 165.
 — leaf, 156, Fig. 43, 158, 161, 165.
 — shoot, 116, Fig. 31, 124, 127.
 — strip, Fig. 92.
 — water in wood, 351.
 acetic acid, 249, 250, 252, 424.
 aceto-carminic, 478, 533, 542.
 achene, 586-9, 592.
 acid, dye, 429.
 — group, 234.
Aconitum, 592.
 acropetal, 387.
Actea, 600, 601, 602.
 actinomorphous, 573.
 adaptation, 143, 560-1.
 adhesion, 557.
 adult, 52, 56, 58-67, 210, 313-26, 421.
æcidia, 451, Fig. 119.
 æcidiospore, 452.
 aerial root, 408.
 aerobic, 276, 464.
Æsculus Hippocastanum, bud scale, 147, 151.
 — hairs, 290.
 — leaf, 165.
 — shoot, 124, 127, Fig. 33.
 — wood, 335.
 aestivation, 557.
Agave, 72.
Agrimonia, 606.
Agropyron repens, 94.
Agrostis stolonifera, 94.
 air, bubble, 177.
 — in intercellular spaces, 207, 209, 210, 213, 222, 339, 376, 400.
Ajuga reptans, 81, Fig. 17.
 albumen cement, 196.
Alchemilla, 605, 606.
 alcohol (see also ethyl alcohol), 277, 465.
 aldehyde, 234, 277.
 alder (*Alnus*).
 aleurone, 39, 254, Fig. 66.
 algae, 6, 191, 419, 421, 500-13.
 alighting platform, 572, 573, 576.
Alisma Plantago-aquatica, 39, Fig. 13, 160, 165, Fig. 45, 499.
 allelomorphous, 548.
Allium Cepa, bulb, 101.
 — mitosis, Fig. 154, 543.
 — seed, 39, Fig. 12.
 — *Porrum*, 101.
Alnus glutinosa, 150, Fig. 87, 428.
 aloe, 93.
Alopecurus pratensis, 562.
 alpine, 79, 574.
Alstræmeria, 377, 545, Fig. 155.
 alternate leaf arrangement, 51, 168.
 alternation of generations, 519, 525.
 — of whorls, 559, 604.
Althæa, 584.
 American aloe, 72.
 amino-acetic acid, 252.
 — acid, 25, 252.
 — group, or amide, 249.
 ammonia, 226, 430.
 ammonium, 193, 429, 430, 442.
 — oxalate, 194.
 — salts, 429, 430.
 amylopectin, 244.
 amylose, 244.
 anærobic, 267, 271, 277, 464.
 anaphase, Fig. 154, 543.
 anatropous, 493, Fig. 135.
 androecium, 469, 557, 558.
 androspore, 537.
Anemone, 45, 486, 600.
 anemophilous, 561.
 angiosperm, 5, 471, 482, 536-8.
 aniline, chloride, and sulphate, 314.
 annual, length increment, 118.
 — meadow grass, 70, 93.
 — plants, 68.
 — ring, 120, 335-42, 342-6.
 annular, 305, 307, 369, 399.
 annulus, Fig. 147, 522.
 ant, 31, 446, 447.
 anterior, 557.
 antero-posterior, 557.
 anther, 471-9, Fig. 124, Fig. 125, 557, 568.
 antheridium, 510-11, Fig. 141, 513-14, Fig. 144, 523, 527.
 anthocyanin, 258.
Anthophora, 581.

Anthriscus sylvestris, 574, Fig. 164.
 anticlinal, 203.
 antipodal, 490, 536.
Antirrhinum, 590, Fig. 168.
 apex (see under root and shoot).
 apocarpous, 471, 557.
 apple (*Pyrus*).
Aquilegia, 600, 601.
Araucaria imbricata, 67.
 archegonium, 515-18, Fig. 145, 523-9,
 Fig. 152, 535.
 archesporium, 489, 522.
Armoracia lapathifolia, 274.
 aromatic, 576.
 arrowhead, 160.
 artichoke, Jerusalem, 83.
Arum maculatum, 576.
 ascomycetes, 467.
 ascospore, 467.
 ascus, asci, 467.
 ash (mineral), 24, 226.
 — (tree) (*Fraxinus*).
Aspidistra, 162.
Aster, 77.
 asymmetric, 173, 492.
 atropous, 492, Fig. 134.
 auricles, 161.
 autoclave, 462.
 autumn wood, 338.
 auxine, 381-3.
 axil, 52, 62, 64.
 axile placentation, 484, 593.
 axis, 51.

BACTERIA, 268, 270-1, 277, 278, 428-9,
 448, 456.
 baking, 466.
 balsam, poplar, 565.
 bamboo, 72, 92.
Bambusa, 72.
 banana, 135, 583.
 banyan, 131, 135.
 barberry, Fig. 15, 66, 450.
 bark, 121, 122, 342, 364-5, 408, 439.
 barley, 240, 287, 465.
 basal, cell, 495, Fig. 137.
 — placentation, 486.
 basic dye, 429, 540.
 — slag, 430.
 basidiomycetes, 454.
 basidiospore, 454.
 basipetal, 159, 383.
 basket willow, 133.
 bast, 121.
 Bateson, 551.
 bean (*Vicia* and *Phaseolus*).
 bee, 565, 568, 569, 570, 572, 576.
 — flies, 581.
 beech (*Fagus*).
 beet, 44, 71, 258.
Bellis perennis, 575.

bent, 94.
 Bentham and Hooker, 598.
 benzaldehyde, 272.
 berry, 593, 600, 602.
Beta vulgaris, 44, 71, 258.
Betula, 126, 335, 364.
 — *pendula*, 122, 335.
 bicollateral, 318.
Bidens, 587.
 biennial, 71, 78.
 bifoliar spur, 531.
 binomial system, 3, 594.
 birch (*Betula*).
 bird, 561, 572, 585.
 bird's nest, 434.
 — orchid, 434.
 biuret, 256.
 blackberry, 66, 587, Fig. 166.
 black, bryony, 159.
 — currant, 133.
 — Italian poplar, 138, 563.
 bladder campion, 590.
 bladderwort, 443-6, Fig. 118.
 bleeding, 413, 415.
 bluebell, 108, Fig. 30, 110, 111.
 body cell, 535.
Boehmeria nivea, 324.
 bog, 441, 443, 446.
 bole, 130, 350.
 bolter, 72.
Bombus, 581.
Bombylius, 581.
 bond, 232.
 borage, 569.
 bordered pit, 314-15, Fig. 78, 343.
 boron, 425, 431.
 Boussingault, 226.
 box, 212.
 bract, 557, 563.
 bramble, 163.
 branch, root, Fig. 105, 387-9, 391, 402.
 — shoot, 62, 136, 137.
 branching, 402, 431.
Brassica campestris, 71.
 — *oleracea*, 71, 154, 260.
 bread fruit, 135.
 brewing, 465.
 broad bean (*Vicia*).
 broad-leaved, 116.
 broccoli, 71.
 broom, 31.
 broomrape, 437.
 Brown, Robert, 184.
 brussels sprout, 71, 154.
 bud, 51, 52, 115, 126.
 — accessory, 65, 151.
 — dormant, 70, 77, 118, 125, 126, 138,
 151.
 — flower, 126.
 — on radial growth, 349-55.
 — on roots, 88, 403.
 — position, 64.

- bud scale, 116, 118, 142-53, Figs. 38-42, 563.
 budding, 133-5.
 — of yeast, 466.
Buddleia variabilis, 577.
 bugle, 81, Fig. 17.
 bulb, 100-13, Figs. 25-30.
 — scales, 100, 105.
 bulbils, 112.
 Buller, R. H., 176.
 bundle, fibro-vascular, 313-14.
 — sheath, 292, 372, 376.
 — vascular, 293-5, 301-12.
 burr-knot, 133.
 bush, 115, 129, 130, 563.
 buttercup (*Ranunculus*).
 — creeping (*R. repens*).
 — meadow (*R. acris*).
 — water (*R. heterophyllus*).
 butterfly, 569, 576, 577.
 butterwort, 441, 443.
Buxus sempervirens, 212.

CABBAGE, 71, 260.
 cacao tree, 120.
 cactus, 267.
 calcium, 423, 424.
 — carbonate, 424, 430.
 — oxalate, 424.
 — pectate, 193, 293, 424.
Callitriche stagnalis, 165.
Calluna vulgaris, 291.
 callus, phloem, 321.
 — wound, 358.
 calories, 269.
Caltha palustris, 482-4, Fig. 128, 486, 492, 592, 600, 601.
 calyx, 469, 557, 593.
 cambium, 121, 134, 308, Fig. 75b, 318, 325-6, 327-31, 336-42, 343, 348-61, Fig. 91, 388, 403-5, 439.
Campanula, 590, Fig. 168.
 campylotropous, 493, Fig. 135, 497.
 Canada balsam, 196.
 Canadian poplar, 563.
 candytuft, 574.
 cane sugar, 185, 239, 420, 479.
Cannabis sativa, 250, 253, 324.
 capitate hair, 443.
 capitulum, 574-5.
Capsella Bursa-pastoris, 68, 78, Fig. 135, 493, Fig. 135, 495-8, Fig. 137, Fig. 169, 593.
 capsule, fruit, 590-1, 600, 602.
 — moss, 6, Fig. 143d, Fig. 146, 518-19.
 carbohydrate, 229-31, 235-49, 358, 437, 468.
 carbon, 24, 226, 232.
 — assimilation, 226.
 — dioxide, 24, 226-30, 233, 267-8, 276-278, 420, 430.
 carboxyl, 234, 249, 250, 252, 253.
 carotin, 259-61.
 carpel, 468, 482-99, Fig. 128, 537, 557, 584, 589-90, 595.
 carpellate, 563, 565-7.
 carrot, 71, 193.
 caruncle, 30.
 Caryophyllaceæ, 484, 485.
 Caspian strip, 396-9, Fig. 109, 412.
Castanea, Fig. 92.
 castor oil (*Ricinus*).
 catalase, 276, 278.
 catalyst, 241, 430.
 catechol, 275.
 catkin, 140, 563-8, Figs. 157-9.
 cauliflower, 71.
 cell, 181, Figs. 48-9.
 — division, 201-8, 383, 497, 540-4.
 — extension, 380, 382.
 — permeability, 442.
 — sap, 184.
 — wall (see also extine, intine), 181, 183, 190, 201, 287, 386, 398, 402, 415.
 cellophane, 246, 286.
 cellulose, 39, 181, 201, 216, 226, 239-41, 245-7, 401-2, 406, 476.
 censer, 590.
Centaurea, 77.
 — *Cyanus*, 575.
 cereal, 46, 425, 427, 449.
 chalaza, 493.
Chamenerion angustifolium, 68, 403.
 chaparral, 164.
 Chaulmoogra oil, 3.
Cheiranthus Cheiri, 292, 603, Fig. 170.
 cherry, 559, Fig. 130, 587, Fig. 166.
 — laurel, 67, 272, 287.
 chess-board diagram, 550.
 chessmen, 552-3.
 chickweed, 70, 73.
Chlamydomonas, 188-90, Fig. 50, 208, 500-2, Fig. 138.
 chloral hydrate, 369.
 chloride, 421.
 chlorine, 420, 425-6.
 chloroform, 272, 279.
 chlorophyll, 251, 259-61, 279, 424.
Chlorophytum, 394.
 chloroplast, 185, 191, 218, 223, 229, 242, Fig. 64, 500, 502, 504-5, 515.
 chlorotic, 424, 431.
 Christmas rose, Fig. 162, 579.
 chromatin, 540, 542.
 chromatophore, 191.
 chromic acid, 293.
 chromosome, 540-6, 551-5.
 Church, A. H., 577.
Circæa lutetiana, 82.
Circium arvense, 82.
 circulation of protoplasm, 183, 243.
 classification, 5, 594-607.
 — of fruits, 583-93.
 claw, 604.

- Claytonia perfoliata*, 162.
 cleavers, 587.
Clematis, 586, Fig. 165, 600, 603.
 climber, 440, 446.
 clone, 74, 75, 80, 85, 133, 539, 544.
 closed vein system, 158.
 clover, 17, 43, 427.
 — red, 569.
 cluster cup, 451.
Cnicus arvensis (*Circium arvense*), 82.
 cob, 35, Fig. 10.
 cobalt chloride, 286.
 cock's-foot grass, 93.
 cocoa, 135.
 coffee, 135.
 cohesion, 557.
 coleoptile, 37, 89, 381.
 coleorrhiza, 37.
Coleus, 258.
 collateral, 318, 399.
 collenchyma, 299-301, Fig. 72, 329.
 collet, 383.
 colour, change, 272-9.
 — of flower, 568, 574, 575, 576.
 — sense, 576.
 coltsfoot, 81, Figs. 18-19.
 columella, 458, 519.
 combustion, 27, 52.
 comfrey, 559, Fig. 156, 569.
 companion cell, 317-22, Figs. 80-1, 339,
 Fig. 89, 356, 376, 405.
 comparative anatomy, 346.
 Compositæ, 298, 574-5.
 compound leaf, 66, 159.
 — umbel, 574.
 conceptacle, 509.
 condenser, 177.
 cone, 527, 529-35.
 conifer, 77, 116, 328.
 conjugation, 459, 467, 501, 505-8.
 connate, 162.
 connective, 471, 472.
 contractile root, 97, 111.
 convergent, 160, 369.
 Convolvulaceæ, 440.
 copper, 260, 425.
 coral root, 434, Fig. 114.
Corallorhiza trifida, 434, Fig. 114.
Corchorus, 324.
 cork, 114, 118, 122, 285, 329, 361-6,
 Figs. 97-8, 403, 407.
 — cambium, 331, 361, 403, 407.
 corm, 98-9, Fig. 24.
 cornflower, 77, 575.
Cornus sanguinea, 118, 146, 150.
 corolla, 469, 557, 569, 572.
 corpus, 204.
 Correns, 551.
 cortex, 112, 119, 294, 296-301, Fig. 72,
 329, 393, 396, 402, 411, 434, 435.
 corticated bulb, 112.
 corymb, 574, 575.
Corypha umbraculifera, 72.
 cotton, 324.
 cotyledon, 10, 23, 31, 32, 37, 53, 55, 89,
 163, Fig. 137, 497-9, 535.
 couch grass, 94.
Crambe maritima, 88.
Cratægus monogyna, 45, Fig. 15, 66, 222,
 283, 587, Fig. 167, 606.
 creeping buttercup (*Ranunculus repens*).
 crenate, 159.
 cress, 386.
Crocus albidiflorus, 98, Fig. 24.
 cross-pollination, 547, 554, 562, 569, 572,
 575, 579, 580, 581, 583.
 crowberry, 291.
 crown (tree), 131, 135, 141, 350.
 Cruciferæ, 484, 593, 603-5.
 crystalloid, 255.
Cucurbita, 318.
 cultivation, 426-31.
 culture, media, 462-4.
 — pure, 464, 466.
 — solution, 422-6.
 — stab and streak, 464.
 currant, black, 133.
 — flowering, Fig. 39, 147.
Cuscuta, 440.
 cuticle, 200, 206, 216, 219, 251, 284, 285,
 289-90, 331, 384, 437, 444, 450.
 "cuticular" layer, 193.
 cutting, 133, 383.
 cycad, 529, 535.
Cycas revoluta, 529.
Cyclamen, 66.
 cyme, cymose, 574, 575.
 Cyperaceæ, 172.
 cypselæ, 578.
 cystine, 256.
 cytase, 241.
Cytisus scoparius, 31.
 cytology, 537, 544.
 cytoplasm, 184, 191, 200, 202, 533.
Dactylis glomerata, 93.
 daffodil (*Narcissus*).
 dahlia, 87.
 daisy, 575.
 damping off, 422.
 dandelion, 88, 574, Fig. 165, 587.
 darkness, 49, 53, 54, 56, 164.
 Darwin, Charles, 2, 142, 442, 581, 595.
Daucus Carota, 71.
 de Bary, 449.
 de Vries, 551.
 decay, 25, 426, 429, 456.
 deciduous, 116, 563.
 decussate, 171, 194, 575.
 definite inflorescence, 574.
 definitive nucleus, 490-1, 494, 536, 538.
 dehiscence, fruit, 498, 584, 590.
 — sporangium, 522.
 — stamen, 477.

- dehydrase, dehydrogenase, 275, 278.
Delphinium, 579-80, Fig. 162, 592, 600, 601.
dentations, 159.
dermatogen, 203, 211, 395.
descaling, 150.
Deschampsia caespitosa, 93.
desert, 71.
development, 143, 154, 575.
dextrin, 240.
diadelphous, 559.
dialyser, 254.
diamond, 234.
diarch, 399.
diastase, 241, 465.
dichasial, 575.
dichotomy, 508.
dicotyledon, 89, 203, 205, 477, 495, 557.
— herbaceous, 77-88.
— leaf form, 155-9, 161-5.
— phyllotaxis, 168-72.
— root, 387, 389-91, 407.
— seed, 8-35.
— shoot structure, 292-347.
— woody, 114-41.
didynamous, 560.
differentiation, 303, 304, 348, 352-6, 416.
diffuse porous, Fig. 87, 338.
diffusion, gaseous, 226.
— in liquid, 18, 419.
Digitalis purpurea, Fig. 156, 560, 570-1, Fig. 160.
dimorphism, 581.
diœcious, 507.
Dionea muscipula, 443.
diploid, 545.
disaccharide, 238.
disease, 123, 422, 448-56, 554.
dissolve, 18.
division, cell, 201, Fig. 56.
— nucleus, 540-6, Figs. 154-5.
dock, 88.
dodder, 440.
dog's mercury, 82.
dogwood, 118, 146, 150.
dominant, 548.
dormant, bud, 70, 77, 118, 125, 126, 138, 151.
— seed, 45.
Doyle, 533.
dropper, 111.
dropwort, water, 87.
Drosera, 441-3.
— *longifolia*, 441.
— *rotundifolia*, 441.
Druce, 598.
drupe, 587, 593.
drupel, 587.
dry, fruit, 584-7, 590-3.
— weight, 18, 19-27, 49-57, 225, 227, 264.
Dryopteris Filix-mas, 520-5, Figs. 147-8.
Dumas, 226.
dung, 429.
dwarf shoot, 126, 531.
EARLY WOOD, 120, 336-8, 343-6.
Eau de Javelle, 248, 402, 478, 482, 495, 515.
Ecballium Elaterium, 585.
ecology, 5:
elder (*Sambucus*).
elm (*Ulmus*).
Elodea canadensis, 227, 242, Fig. 64, 259.
embryo, 23, 33, 37, 44, 45, 439, 495-9, 525, 528, 535, Fig. 135, 538, 583.
— cell, 495.
— sac, 490-5, 536, 537.
Empetrum nigrum, 291.
enchanter's nightshade, 82.
endocarp, 587.
endodermis, 296, Fig. 107, 395-9, Fig. 109, 396-9, 406-8, Fig. 112, 411-12.
endosperm, 402.
endosperm, 33-4, 37, 39, 41, 254, 465, 494-5, 497-9, 535, 538.
energesis, 271.
energy, 25, 262, 269, 271, 278-9.
entire leaf margin, 161.
entomophilous, 561.
enzyme, 241, 253, 272-9, 447, 455, 457, 465.
cosin, 429.
ephemeral, 70, 73.
epicarp, 587.
epicotyl, 12, 56, 144.
epidermis, 114, 203, 213-24, 294, 296-311, Fig. 72, 370, 372, 437, 442, 450.
epigeous, 13, 30, 34, 41, 55, 56, 110.
epigynous, 487, 559.
Epilobium, 591.
epipetalous, 557, 559-60.
epiphyte, 446.
equatorial plane, 542, 552.
Equisetum, 526, 536.
Erica, 291, 477.
Ericaceæ, 432, 478.
ester, 249, 250.
etheral oil, 576.
ethyl, acetate, 250.
— alcohol, 250, 279, 466.
etiolated, 54, Fig. 14.
eu-meristem, Figs. 53-4, 200-5, 211, 402.
eu-meristematic, 294, 295, Fig. 99.
Euphrasia, 436.
evaporation, 149, 280-91, 415-17.
evergreen, 116.
evolution, 2, 142, 595-6.
exodermis, Fig. 108, 405, 408, 426.
exogenous, 402.
extension growth, 115, 119, 123, 208, 416.
extine, 476, 478, 479.
extra-floral nectary, 33.
extrorse, 558.

eyebright, 436.
eye-piece, 175.
eye-spot, 188.

FACULTATIVE, 449, 455.

Fagus sylvatica, 148, 435.

— bud scale, 148.

— evaporation from, 290.

— fruiting, 129.

— hairs, 290.

— layering, 131, Fig. 34.

— shoot, 124, 126.

family, 449, 601-7.

fat, 25, 216, 249-51, 361, 365, 398.

fatty acid, 25, 249, 250.

Fehling's solution, 236, 247.

female prothallium, 535-7.

fermentation, 277, 461, 465-6.

fern, 5, 520-5, 536.

fertile, pollen, 565.

fertilisation, 8, 74, 492, 494, 499, 518,

525, 528, 535, 538, 544, 554.

Fibonacci series, 169.

fibre, bast (sclerenchyma), Fig. 77, 322-325, 339-42, Figs. 89-90, 405.

— wood, Fig. 77, 316, 331-3, Fig. 85, 336.

fibrous, layer, 476, Fig. 126.

— root, 93, 387-8.

fibro-vascular, 313.

Ficus benghalensis, 131.

field, speedwell, 81.

— thistle, 82.

filament, alga, 191-3, 502-8.

— fungus, 393.

— stamen, 471, 477, 559, 562, 572.

file meristem, 207.

filial, 548.

fixing fluid, 195.

flagellum, flagella, 188-90, 500-4, 511-513, 515, 523, 527, 531.

flax, 324.

flies, 463, 576.

floating leaves, 165.

flora, 594, 597-8, 607.

floral, diagram, 556-8, Fig. 156, 603, Fig. 170.

— formula, 558-60, 604.

flower, 29, 62, 468-71, 556-83.

— bud, 126.

— production, 126, 141.

— stalk, 101, 557.

flowering, bud, 127.

— currant, Fig. 39, 147.

foliar scale, 146.

follicle, 592, 600.

food, 24.

— conduction, 359.

— to root apex, 401-2.

foot, 518, 525, 528.

foraminifera, 555.

forest, 137, 350, 391, 430, 432.

formaldehyde, 233-6, 264.

formic acid, 233, 250.

Forsythia, 162.

foxglove, 560, 570-1, Fig. 160.

foxtail grass, 562.

Fragaria vesca, 75, 80, Fig. 16, 81, 587,

Fig. 166, 605.

Fraxinus excelsior, bud scale, Fig. 40, 147.

— fruit and seed, 33-4, Fig. 9, 35, 55, 129, 586.

— leaf, 66, 156, Fig. 43.

— phloem, 356.

— shoot, 124.

— strip, Fig. 91.

— wood, Fig. 87.

free, central placentation, 485, Fig. 129, 559, 590.

— stamens, 557.

French bean, 13.

fresh weight, 19-22.

frond, 508, 520.

frost, 43, 76, 114, 125, 127, 151.

fructose, 238.

fruit, 8, 29, 33, 62, 498, 583-93.

— wall (pericarp).

fuchsin, acid, 450.

— basic, 467.

Fucus, 508-13, Figs. 141-2.

— *serratus*, 509.

— *vesiculosus*, 509.

fungus, fungi, 6, 448-67.

— diseases, 42, 87.

— mycorrhiza, 393, 432-5.

funicle, 8, 30, 41, 493, 494, 537.

fusiform, 348, 349, Fig. 91.

fusion, 539, 544, 551.

Galium Aparine, 587.

gall, 583.

gametangium, 459, 460.

gamete, 501, 504, 505, 519, 544, 549.

gametophyte, 519, 525, 527-9, 538.

gamopetalous (sympetalous), 559.

gamoepaly, 572.

gel, 542.

gene, 548, 551, 552, 554.

generative nucleus, or cell, 478, 479, 533, 535, 537.

genetic, 546.

gentian violet, 189.

genus, genera, 3, 449, 557, 597, 600-1.

geophilous, 79, 112.

geotropism, 379.

geranium, 584.

germ, pore, 452, 479.

— tube, 450.

germination, 14-27, 28-48.

Geum, 587.

gibbous, 603.

Ginkgo biloba, 529.

girdle scar, 116, 126.

girdling, 354-5.

- girth growth, 76, 119-23.
glandular hair, 437, 442, 446.
Glechoma hederacea, 81.
globoid, 254-5.
glucose, 236-40, 465, 466.
glucoside, 272.
glume, 562.
gluten, 466.
glycerol, 25, 249, 466.
glycine, 258.
— glycol, 252.
glycogen, 465.
gooseberry, 66, 593.
gorse (*Ulex*).
grafting, 133-5, Fig. 35.
grain, aleurone, 254.
— cereal, 35, Fig. 10, 254.
— starch, 243-7.
graphite, 234.
grass, coleoptile, 381.
— family, 562.
— growth, 92, 93, 94.
— leaf, 162, 369.
— root, 387.
grassland, 436.
gravity, 165, 379.
green, light or fast, 302.
— manuring, 428.
grey poplar, 565.
ground ivy, 81.
groundsel, 59-63, 70.
growing apex, root, 384-7, 394, 401-2.
— — shoot, 155, 196-208, Figs. 52-4,
367-9, Fig. 99.
— regions, 52, 421.
growth, 201, 207, 381, 394, 443.
— curvature, 379-82.
— extension, 119, 123, 207, 416.
— in dark, 53-6.
— radial, 76, 119-23.
— rhythm, 153.
guaiacum, 273.
guard cell, 219-24, Figs. 62-3, 371, Fig.
100.
guelder rose, 127.
gymnosperm, 5, 115, 134, 529-36, 537.
gynæceum, 471, 484, 557, 563, 604.
gynospore, 537.
- HABER**, 430.
Haberlandt, 281, 395.
hæm, 279.
hæmoglobin, 424.
hair, capitate, 444.
— flower, 569.
— glandular, 437, 442.
— root, 386-7, 411.
— seed, 591.
— shoot, 290.
— staminal, 541.
Hale, Stephen, 281.
Hanstein, 204.
- haploid, 545.
hard seeds, 17, 43.
hardwood, 116, 121, 135, 327-42, 343-7,
391.
Hartig, Th., 349.
haulm, 87.
haustorium, 435, 436, 437: 440-1, 450,
Fig. 119.
haw, 587.
hawthorn (*Crataegus*).
Hayward, 598.
heartwood, 346.
heath, 291, 477.
heather, 440.
Hedera Helix, 163.
Helenium, 64, 161.
Helianthus, 228, 559.
— *annuus*, 29-30, Figs. 5-6, 35, 293-326,
Figs. 71-2, 74-9.
— *decapetalus*, 83.
— *tuberosus*, 83.
helicoid, 575.
Helleborus, 600, 601.
— *niger*, 579, Fig. 162.
hemp, 250, 253, 324.
Hendrickson, 282.
Heracleum Sphondylium, 576.
herbaceous, 414, 603.
— dicotyledon stem structure, 292-326.
— monocotyledon structure, 367-78.
— perennials, 76-113.
herbarium, 597.
heredity, 539-55.
heterosporous, 536.
heterostylic, 581.
hexose, 235-9.
hilum, seed, 9, 31.
— starch grain, 244.
hinge cell, 372.
hip, 587.
Hitchcock, 597.
hogweed, 576.
holdfast, 508.
holly, 67, 116, 146, 163.
hollyhock, 584.
homology, 145, 536-8.
homosporous, 536.
honesty, 593, Fig. 169.
honeysuckle, 162, 577.
Hooke, R., 183.
horizontal stem, 79, 165.
hormone, 381.
horse chestnut (*Æsculus*).
horse-radish, 274.
horse-tail, 526, 536.
host, 435, 437, 438, 439, 440-1, 449.
house leek, 78.
humble-bee, 569, 570, 571, 579-80, 581.
humming bird, 561, 572.
humus, 426, 457.
Huxley, 183.
Hyacinthus, 110, 387, 474.

hybrid, 548, 565.
 hydrochloric acid, 194.
 hydrocyanic acid, 272.
 hydrogen peroxide, 275, 276, 278, 279.
 — acceptor, 277, 278.
 hydrolysis, 24, 239, 253.
 hydroxyl, 235, 240.
 hygroscopic, 584.
Hypericum, 590.
 hypha, hyphæ, 393, 434, 450-60.
 hypocotyl, 12, 31, 56, 383.
 hypogeous, 13, 34, 39, 41, 46, 55, 56.
 hypogynous, 487, 557, 604.

Iberis amara, 574.
 identification, 556.
Ilex Aquifolium, 67, 116, 146, 163.
 imbricate, 557.
 indefinite, flower parts, 558.
 — inflorescence, 574.
 indehiscent, 584.
 Indian, ink, 379, 413, 414, 416.
 — Lotus, 47.
 individual, 68-75, 191, 539.
 indoleacetic acid, 383.
 indusium, 520, Fig. 147, 529.
 inferior, 487, 559.
 inflorescence, 79, 96, 556, 557, 562, 563,
 574-5, 580.
 — axis of, 557, 573.
 — scar, 128.
 inheritance, 546, 552, 553.
 initial, cambial, 330.
 — fusiform, 348, Fig. 91.
 — ray, 330, 338.
 — root, 131.
 injection (of vessels), 413-15, 416.
 insect, 441, 479, 517, 561, 567-83.
 insectivorous, 441-7.
 insertion (leaf), 156.
 integument, 489-94, 498, 529, 531, 537.
 intercellular spaces, 193, 206, 209, 213,
 218, 219, 221, 284-6, 292, 338, 376,
 509.
 internode, 51, 91, 208.
 — extension of, 51, 207.
 — length, 59, 90.
 — structure, 293-312, 313-26, 374-6.
 interphase, 540, 543, 544.
 intine, 476, 479.
 introrse, 604.
 iodine, test for cellulose, 181.
 — — — glycogen, 465.
 — — — protein, 183, 188, 254-5.
 — — — starch, 36, 119, 229.
Iris, 94, Fig. 22, 387, 406-7, Fig. 112, 590,
 Fig. 168.
 — *germanica*, 94.
 iris diaphragm, 177.
 iron, 279, 423, 424.
Isatis tinctoria, 605.
 islets, vein, 158, 211.

isomer, 236.
 ivy, 163.

JAMES and Clapham, 577.
 Jameson, H. G., 598.
 Java, 151.
 Jerusalem artichoke, 83.
 jute, 324.
 juvenile foliage, 165.

KALINIT, 431.
 karyokinesis, 540.
 keck, 574.
 keel, 570, 572
 kernel, 535.
 ketose, 238.
 "keys," 129.
 kidney bean, 13.
 klinostat, 379.
 knot, 136.
 Koch, Robert, 462.
 kohl-rabi, 71.

LABIATÆ, 171, 584.
Laburnum anagyroides, 147, 346.
 lacquer, 274.
 lactic acid, 277.
Lagenia sulcata, 555.
 lamina, 64, 90, 155, 156.
Lamium album, 572-3, Fig. 161.
 larch, 116.
Larix decidua, 116.
 larva, 568, 583.
 late wood, 120, 338, 343-6, 355.
 laterite, 431.
 latex, 274.
Lathraea Squamaria, 243, 436-7, Fig. 116.
 leaf, 51, 58, 64.
 — abscission, 365.
 — arrangement, 51, 168-73.
 — base, 147, 161.
 — buds, 126.
 — expansion, 114.
 — fall, 118, 149.
 — gap, 294.
 — growth, 51.
 — insertion, 156.
 — margin, 161.
 — primordia, 153, 155, 156, Fig. 43, 162,
 170, 205, 211-12, 294-6, Fig. 70,
 367, Fig. 99, 482.
 — scar, 67, 117.
 — shape, 66, 142, 154-67, Figs. 44-5.
 — sheath, 89, 90, 162.
 — size, 59, 90.
 — structure, 211-24, 369-74.
 — trace, 293-4, 310-12, Figs. 75-6, 328,
 368, 375-8.
 leek, 100.
 legume, 593.
 Leguminosæ, 14, 427, 428, 440, 572, 593.
 lenticel, 119, 362-3, Fig. 97, 408.

- Lepidium sativum*, 386.
 leucoplast, 242.
 Liebig, 226.
 life cycle, 63, 68-75.
 light, 49, 57, 126, 164, 380-2.
 light green, 198.
 lignification, 305, 314, 476.
 ligule, 90, 101, 162.
Ligustrum vulgare, berry, 184, Fig. 49.
 — bud scale, 146.
 — growth, 115, 123.
 — leaf, 66, 156, Fig. 43.
 — shoot apex, 195, Figs. 52-4, 294-5, Fig. 70, 302.
 lilac, bud scale, 146, Fig. 38.
 — habit, 115, 123, 127.
Lilium, 112, 172, 472, 475, 479, 493, 544.
 lily (*Lilium*).
 limb, 604.
 lime (*Tilia*).
Limonium sinuatum, 64.
Linaria, 569.
 ling, 291.
 Linnæus, 2, 595.
Linum usitatissimum, 324.
 Lister, 462.
 liverwort, 6, 519.
 Lloyd, 506.
 lobed leaf, 159.
 loculicidal, 590.
 loculus, 471, 484, 487.
 lodicule, 562.
 loganberry, 163.
Lolium perenne, 562.
 long shoots, 125.
Lonicera Caprifolium, 162.
 — *Periclymenum*, 577.
 loosestrife, 325.
 Lorantheæ, 439.
Lunaria, 593, Fig. 169.
 lupin (*Lupinus*).
Lupinus polyphyllus, 559, 571-2, Fig. 160.
Lysimachia vulgaris, 325, 326.
- MACERATION**, 194, 201, 212, 214, 293, 331.
 MacGregor Skene, 576.
 Magdala red, 292.
 magnesium, 259, 262, 279, 423-4.
 magnification, 176, 187.
Mahonia Aquifolium, 450.
 maiden-hair tree, 529.
 maize (*Zea*), 281.
 malaria, 455.
 male, cell or nucleus, 480, 492, 529, 535, 537, 538, 549.
 — fern, 520-5.
 — gametophyte, 536, 537.
 mallow, 584.
 maltase, 241.
 maltose, 239-41, 466.
- Malus pumila* (apple), 606.
 — bud and bud scale, 126, 147, Fig. 41.
 — evaporation from fruit, 284.
 — fruit, 584, 589, Fig. 167.
 — fruiting, 129.
 — grafting, 133.
 — stem-structure, 328-35.
Malva, 584.
 manganese, 423, 424, 431.
 mangroves, 46.
 manure, 426-31.
 mâquis, 164.
 marginal placentation, 484.
 "mast," 129.
 maturation, 45.
 Maximov, 291.
 meadow buttercup, 577.
 meadow grass, annual, 70, 93.
 Mediterranean, 113.
 medullary (see ray).
 megasporangium, 528, 529, 535, 537.
 megaspore, 489-90, 527-9, 535, 536, 537.
 — mother cell, 489, 535, 536.
 megasporophyll, 529, Figs. 151-2.
 meiosis, 544-6, Fig. 155.
Melampyrum, 436.
 Mendel, Abbé, 546.
 Mendelism, 546-54.
Mercurialis perennis, 82.
 meristem (see also eu-meristem and vacuolating meristem), Figs. 52-3, Fig. 55, 200-8, 303, 308, 473, 495.
 mesocarp, 587.
 mesocotyl, 38.
 mesophyll, 214-19, Figs. 60-1, 369-72.
 metabolism, 225-75, 231.
 metaphase, 542, 543, 545.
 metaphloem, Fig. 71, 317-22, Fig. 81, 376, 403.
 metaxylem, 308, Fig. 71, Figs. 74-5, Fig. 77, 399-400, 403, 414.
 methane, 232.
 methyl, 235, 260.
 methylation, 235.
 methylene blue, 192, 194, 278, 429, 467, 480.
 michaelmas daisy, 77.
 micro-organism, 426-9.
 micropyle, 10, 30, 41, 489, 493, 495, 529, 531, 533, 535, 536, 537.
 microscope, 175-9, Fig. 47.
 microsporangium, 526, 529, 531.
 microspore, 527-8, 536-7.
 microsporophyll, 529, Fig. 150.
 microtome, 196.
 middle lamella, 194, 293, 333, 424.
 midrib, 154, 156, 161, 162, 373.
 Millon's reagent, 256.
 mineral ash, 23-4, 226.
 mint, 456.
 mistletoe, 439.
 mitosis, 540-4, Figs. 154-5.

- Mnium hornum*, 513-19, Fig. 143, Figs. 145-6.
 monadelphous, 559, 572.
 monocarpic, 68, 72.
 monochasial, 575.
 monocotyledon, 72, 77, 115, 134, 477, 495, 557.
 — leaf form, 155, 160, 162.
 — phyllotaxis, 168, 172.
 — polycarpic, 89-113.
 — roots, 387, 388, 407, 408.
 — seed, 35-41.
 — shoot structure, 367-78, Figs. 99-104.
 monoecious, 507.
 monopodial, 124, 128.
 monosaccharide, 238.
Monotropa Hypopitys, 434.
 moorland, 288, 432, 441.
Morus, 164.
 moss, 6, 513-19, Figs. 143-6.
 moth, 569, 572, 576.
 motility, 188-90, 529-30.
 motor cell, 372.
 mould, 457.
 mountain ash, 147.
 mucilage, 191, 193, 434, 502, 508, 509, 510, 511, 517.
Mucor, 457-60, Figs. 121-2.
 — *hiemalis*, Fig. 122.
 — *Mucedo*, 457, Fig. 121.
 mulberry, 164.
 muskeg, 446.
 mustard, white, 380, 386, 428.
 mycelium, 393, 450-60.
 mycorrhiza, 391-3, Figs. 106-7, 432-5, Fig. 113.
 "NAKED" buds, 150.
Narcissus Pseudo-Narcissus, bulb, 101-5, Figs. 25-8.
 — — flower, 487-8, Fig. 131, 559.
 — — leaf, 92.
 nasturtium, garden, 52, 161, 287.
 natural selection, 142, 575.
 neck, 515, Fig. 145, 523-5, 527.
 — canal, 515, 517, 523-5.
 — canal cells, 515, 517.
 nectar, 450, 455, 469, 570, 572, 581.
 nectary, 33, 567, 569, 570, 579.
 needle-leaved, 116.
Nelumbium speciosum, 47.
Neottia Nidus-avis, 434, Fig. 113.
Nepenthes, 446, 447.
 nettle, 259.
 New Zealand, 165.
Nicotiana, 576.
Nigella, 600, 601, 602.
Nitella, 419.
 nitrate, 226, 265, 421, 429.
 nitrite, 429.
Nitrobacter, 429.
 nitrogen, 247, 421, 423, 425, 426-30, 441.
Nitrosomonas, 429.
 node, 51.
 nodule, 427-8.
 non-endospermic, 34, 41.
 Nottingham catchfly, 576.
 nucellus, 488, 491, 493, 494, 495, 533-8.
 nuclear, membrane, 540.
 — spindle, 542.
 nucleic acid, 257, 540.
 nucleolus, nucleoli, 540.
 nucleo-protein, 540, 544.
 nucleus (see also reduction division), 184, 191, 198, 200, 202, 206, 458, 459, 460, 474, 479, 490-2, 500, 501, 539-46, Figs. 154-5, 551-5.
 nutlet, 584.
 OAK (*Quercus*).
 oat, 381-2, 389.
 obdiplostemonous, 559.
 objective lens system, 175.
 obligate, 449, 455.
Odontites, 436.
Enanthe crocata, 87.
Oenothera, 590.
 offset, 78.
 oil, 177, 200, 249-51, 255, 267.
 onion (*Allium*).
 oogonium, 509, 511, Fig. 142.
 oospore, 492, 495, 498, 513, 518, 525, 528, 539, 551.
 optical section, 178.
 orange, 563, 564, 583.
 orchid, 408, 432-4.
 organic compounds, 24, 25, 52, 57, 225, 426, 428, 429, 432.
 Origin of Species, 2, 595.
Orobanche, 437.
 orthostichy, 168.
 orthotropous, 492, Fig. 134.
 osier, 133.
 osmic acid, 189.
 osmometer, 412.
 osmosis, osmotic pressure, 18, 411-12, 415, 416, 419, 479.
 ostiole, 509.
 ovary, 383, 471, 484-9, Fig. 129, 498, 537, 584, 593.
 ovulate, 531-2, Fig. 151.
 ovule, 468, 471, 481, 484-6, 488-94, Figs. 133-6, 497-8, 529, 531, 533, Figs. 151-2, 534, 535, 536, 537.
 ovum, 490-2, 494, 509-13, Fig. 142, 523-525, 529, 536, 537, 549.
 oxidase, 274, 276, 425.
 oxidation, 26, 233, 269, 424.
 oxidising enzymes, 272-9.
 oxygen, 229, 261, 267-8, 339, 376, 420, 465.
 PALISADE mesophyll, 214, 218.
 palm, 72, 92, 93, 160, 388.

- palmate, 158, 161.
 panicle, 574.
 pansy, 569.
Papaver, 590.
 pappus, 587.
 paraffin wax, 195.
 parallel venation, 160, 369.
 parasite, 434, 435-41, 448-56.
 parenchyma, 210, 294, 298-9, Fig. 72, 370, 405.
 — phloem, 317-22.
 — ray, 333-4, Fig. 86.
 — wood, 316, Fig. 77, 333-4, Fig. 86.
 parietal, 484, 565, 573, 604.
 parsnip, 71.
 partial parasite, 435-6.
 passage cell, 407, 409.
Passiflora, 162.
 passion flower, 162.
 Pasteur, Louis, 461-2.
 pasteurisation, 461.
Pastinaca sativa, 71.
 pathogen, 448.
 pea (*Pisum*).
 peat, 47, 446.
 pectic acid, 193.
 pectin, 192, 204, 301, 476, 479, 480.
 pedicel, 557, 574.
Pedicularis, 436.
 peduncle, 557.
Pelargonium, 286, 416, 584, Fig. 164.
Pellionia Daveauana, 243, Fig. 64.
 peltate, 161.
 pendulous, catkin, 565.
 — ovule, 486, 560.
 pentarch, 403.
 peptase, 253.
 peptone, 25.
 percentage, germination, 42.
 — purity, 42.
 perennial, 68.
 — cornflower, 77.
 — herbaceous, 76-113.
 — monocarpic, 72.
 — rye grass, 562.
 — sunflower, 83.
 — woody, 77, 114-41.
 perforation (of vessel), 306, 316.
 perianth, 470, 489, 563.
 pericarp, 29, 39, 44, 45, 498, 584, 587, 590, 593.
 periclinal, 203.
 pericycle, 395, 399, 402, 405, 407.
 periderm, 361.
 perigynous and perigyny, 487, 559, 605.
 periodicity, 129, 152.
 peristome, 519, Fig. 146.
 periwinkle, 325.
 permeability, 442.
 peroxidase, 274, 276.
 petal, 469, 557.
 petiole, 53, 64, 161.
 Petri dish, 462-4.
 Pfeffer, 281.
 phærophytin, 260.
Phajus grandifolius, 243, Fig. 64.
Phaseolus, 17, 31.
 — *multiflorus*, 12-13, Fig. 4, 35, 410, 570.
 — *vulgaris*, 13, 35.
 phellogen, 331, 361, 403, 407.
Philadelphus coronarius, 123, 130.
 phloem (see also protophloem, meta-phloem), 121, 317-22, Figs. 79-81, 339, 356-7, 358-61, 372, 375-6, 399, 400, 437, 441, 520.
 phloroglucin, 314, 476.
Phlox, 326.
 phosphate, 265, 277, 430, 431.
 phosphorus, 257, 423.
 photosynthesis, 226-31, 258-65.
 phototropism, 380-2.
 phyllotaxis, 168-73, Fig. 46, 195, 469, 575.
 phytol, 260.
Picea Abies, 66.
 pigment, 184, 258-62.
 piliferous layer, 395, 405.
 pin-eyed, 580-1.
 pin mould, 457.
 pine (*Pinus*).
 pineapple, 253.
Pinguicula, 441, 443.
 pinnate, 159, 520, 529.
Pinus sylvestris, Fig. 150, 531-6, Figs. 151-3.
 — habit, 531.
 — mycorrhiza, 391, Figs. 106-7.
 — reproduction, 531-6.
 — wood, 343-6.
 — *Pinea*, 535, Fig. 153.
Pisum sativum, 14, 19, 35, 253, 267, 270.
 — Mendelism, 547-51.
 pit, 216, 301, 314-17, Fig. 78.
 — bordered, 316, 331, Fig. 85, 343.
 — canal, 316, 333, Fig. 89.
 — fibre, 331-3.
 — membrane, 316, 333.
 — prop, 345.
 — ray, 334.
 — simple, 325, 335.
 — vessel, 333-5.
 — wood parenchyma, 334.
 pitcher, 446-7.
 pith, 114, 294, 329.
 pitted, 314, Fig. 77, 369, 399, 414.
 placenta and placentation, 484-93, 537, 565, Fig. 158, 604.
 plane, 122.
 planks, 136.
Plantago, 590.
 plasmodema, 201, 204, 316.
 plasmolysis, 186, Fig. 49, 353, Fig. 94, 419, 420.

plasmolysis, recovery from, 419.
 plasticine, 202.
 plastid, 242, Fig. 64, 244.
 plastoehrone, 173.
Platanus orientalis, 122.
Pleurococcus, Fig. 50, 190, 207, 208.
 plum, 587.
 plumular hook, 54, Fig. 14, 380.
 plumule, 10, 14, 20, 31, 39, 46, 55, 497.
Poa, 456.
 — *annua*, 70, 93, Fig. 105.
 poisoning, 273.
 polarising microscope, 245.
 pole of spindle, 542.
 pollen grain, 8, 471, 473, 474, 476, 478–
 480, Fig. 125, 529, Fig. 150, 533,
 536, 537, 562, 570.
 — mother cell, 474, 489.
 — sac, 471, 473, 474, 476, 477, 531.
 — tube, 479–81, Fig. 136, 529, 533, 537.
 pollination (see also cross-pollination,
 self-pollination), 479, 533.
 — drop, 533.
 — mechanism, 559, 561–83.
 polyarch, 399.
 polycarpic, 68.
 Polygonaceæ, 88.
Polygonatum multiflorum, 97, Fig. 23.
Polygonum, 486, 492, 544.
 — *divaricatum*, 489–91, Figs. 133–4.
 polymerisation, 234.
 polypetalous, 557.
 polysepalous, 557.
Polytrichum, 515, Fig. 144.
 pome, 589, 593.
 poplar (*Populus*).
 poppy, 590.
Populus, 122, 125, 126, 141, 563–5, Figs.
 157–8, 591.
 — *candicans*, 565.
 — *canescens*, 565.
 — *serotina*, 138, 563.
 — — var. *aurea*, 563.
 porometer, 222, 284.
 porous dehiscence, anther, 477.
 — — fruit, 589, Fig. 168.
 posterior, 557.
 potassium, 420, 423, 426, 427, 431.
 potato (see also *Solanum tuberosum*), leaf,
 64, 161, 365.
 — starch in tuber, 242, 245, Fig. 65.
 — tuber and clone, 75, 83–7, Figs. 20–1,
 365.
 potometer, 283, Fig. 69.
 prickles, 66.
 primary, ray, 307.
 — tissue, 308, 403.
 — wall, 304, 316, 333, 414.
 — xylem, 308–12.
 primordium, leaf, 153–6, Fig. 43, 162,
 170, 205, 294–6, Fig. 70, 367, Fig. 99,
 482.

primrose (*Primula*).
Primula, 485, Fig. 129, 569.
 — *vulgaris*, 580–3, Fig. 163.
Primulacæ, 484, 485, 604.
 privet (*Ligustrum*).
 proboscis, 580, 581.
 procambium, Fig. 54, 294–6, Fig. 73,
 301, 306, 308.
 propagation, vegetative, 74, 78–88, 365.
 prophase, 540, 541, 543, 545.
 prophyll, 557, 559.
 protandrous and protandry, 562, 570,
 580.
 protease, 253, 442.
 protein, 24, 57, 183, 248, 251–7, 265, 279,
 421, 423, 439, 542, 555.
 prothallial cell, 537.
 prothallium, 523–6, Fig. 148, 527, 535,
 536, 537.
 protoderm, 395.
 protogynous and protogyny, 562, 579.
 protophloem, 302–4, Fig. 73, 329, 376,
 399.
 protoplasm, 183, 201, 243, 279.
 protoplasmic connections, 201, 204, 316.
 protoplast, 190, 201, 415, 500–2.
 — in vessel, 353, 421.
 protoxylem, Fig. 73, 304–7, Fig. 74, 369,
 372, 374–5, 399, 402.
 — cavity, 372, 374.
 protozoa, 428, 455.
 pruning, 122, 126.
Prunus, 587, 605, 606.
 — *Laurocerasus*, 67, 272, 287.
 — *spinosa*, 66.
 prussic acid, 272.
Puccinia graminis, 449–56, Fig. 120.
 — *Menthe*, 456.
 — *Poarum*, 456.
 — *Winteriana*, Fig. 119.
 putrefaction, 456–7, 461.
 puzzle monkey, 67.
 pyrenoid, 188.
 pyrogallic acid, 227, 274.
 pyruvic acid, 277, 278.
 pyxis, 590.
Quercus, abscission, 140, Fig. 37.
 — bud scale, 148.
 — cork, 122.
 — phloem, 356.
 — shoot, 124.
 — wood, 335, 343–6.
Quercus Robur, 140.
 — *petraea*, 140.
 — *suber*, 365.
 RACEME and racemose, 574, 575.
 rachis, 83.
 radial, growth, 119–21, 348–57.
 — root structure, 399.
 radicle, 10, 14, 20, 496–7, 535.

- radish, 71.
 ramie, 324.
 Ranunculaceæ, 486, 577, 592, 601-3.
Ranunculus, 476, 477, 486, 557-8, Fig. 156,
 Fig. 162, 592, 600, 601.
 — *acris*, 577.
 — *Ficaria*, 469.
 — *heterophyllus*, 165.
 — *repens*, 74, 79, 395, Figs. 108-10, 403,
 406, 598.
 — species, 599-600.
Raphanus sativus, 71.
 raphe, 493.
 raphides, 424.
 raspberry, 66, 163, 587.
 ray, 121, 307, 330, 338-42, Figs. 88-90,
 343-6, 405.
 — initial, 330, 338.
 — parenchyma, 333-4, Fig. 86.
 ray floret, 575.
 receptacle, 468, 487-8, 557, 587, 590.
 recessive, 548.
 recombine, 553.
 reducing properties, 233.
 reduction, 233.
 — division, 474, 477, 489, 507, 519, 522,
 535, 544-6.
 redwood, 130.
 refractive index, 178, 180.
 regeneration, cambium, 358.
 — cork, 285.
 Regnault, 227.
 replum, 484, 593, 604.
 reproductive, characters, 598-605.
 — phase, 63, 68, 163, 468.
 resin canal, 342.
 respiration, 26, 264-79.
 response, 381, 443.
 resting phase, 540.
 reticulate, 307, Fig. 77, 372, 374.
 Rhinanthæ, 435.
Rhinanthus minor, 435-6, Fig. 115, 591.
Rhizobium leguminosarum, 427.
 rhizoid, 523.
 rhizome, 81, 94, Fig. 22, 380, 434, 437.
 rhododendron, 146, 288, 477, 478, 590.
 rhythm of growth, 153.
 rib meristem, 207.
Ribes, Fig. 46.
 — *nigrum*, 133.
 — *sanguineum*, Fig. 39, 147.
 — *uva-crispa*, 66, 593.
Ricinus communis, aleurone grain, 254,
 Fig. 66.
 — — fruit and seed, 30-3, Figs. 7-8, 55.
 — — leaf, 161.
 ring porous, Fig. 87, 338, 343, 345, 352,
 414.
 ringing, 358-9.
 root, 14, 20, 58, 97, 111, 359, 408.
 — apex, 383, 387, 394-5.
 — cap, 384, 394.
 root, direction of growth, 379-80.
 — function, 410-31.
 — hair, 386-7, 411.
 — initial, 131, Fig. 105, 387-9, 435.
 — nodule, 427-8.
 — parasite, 435-9.
 — pressure, 413, 415.
 — structure, 383, 409.
 — system, 93, 125, 282, 383-93, 426, 435.
 root crop, 427.
 root-stock, 78, 87, 130.
Rosa, 66, 222, 285, 559, 587, Fig. 167,
 606.
 Rosaceæ, 587, 605-7.
 rose (*Rosa*).
 rose bay willow herb, 68.
 rosette, 78, 163.
 rotation, crops, 427.
 — protoplasm, 243.
 Rothamsted, 47.
 rubber, 135, 274.
 rubbing, 138, Fig. 36.
Rubus, 66, 163, 587, 606.
Rumex, 486, 492.
 — *Acetosa*, 161, 492.
 — *Acetosella*, 88, 403, Fig. 132.
 runner, 79, 80, Fig. 16, 380.
 rust fungus, 448-56, Figs. 119-20.
 ruthenium red, 192, 194.

Saccharomyces, 463-7.
 Sachs, 228, 281, 351.
 safranin, 198, 302.
Sagittaria sagittifolia, 160, 165.
 Salicaceæ, 563.
Salix, 118, 131, 225, Fig. 93, 353, 359,
 Fig. 96, 407, 563, 565-8, Fig. 159,
 591.
 — *Caprea*, 133, 565, Fig. 159.
 — *purpurea*, 568.
 — *viminalis*, 133, 141.
 saw, 133, 565, Fig. 159.
 salts, entry into root, 419-21.
 — supply to plant, 421-31, 441.
 samara, 586.
Sambucus nigra, cork, 361, Fig. 97.
 — — habit, 130.
 — — leaf, 66.
 — — lenticel, 119, 362-3, Fig. 97.
 sap, 376, 437.
 — stelar, 411, 412.
 — tracheal, 411.
 sapling, 130, 141.
 saprophyte, 434, 456-67.
 sapwood, 446, 447.
Sarothamnus scoparius, 31.
Sarracenia, 438, 439.
Sauromatum guttatum, 97.
Saxifraga, 78.
 scaffold, branch, 141.
 — root, 390, 391.

- scalariform, 307, 314, Fig. 77, 369, 372, 399.
 scale, bud, 116, 118, 142-53 Figs. 38-42.
 — bulb, 100, 105.
 — cone, 533.
 — double, 531.
 — flower, 569.
 — foliar, 146.
 — leaves, 12, 51, 53, 80, 100, 101, 107, 436-8, 531.
 — scar, 116.
 — stipular, 148.
 scar, branch, 123, 137, Fig. 36.
 — girdle, 116.
 — inflorescence, 110, 127, Fig. 33, 128.
 — leaf, 66, 117.
 — scale, 116.
 scarification, 43.
 scarlet runner bean, 12-13, Fig. 4, 410, 570.
 scattered bundles, 378.
 scent, 574, 576.
 schizocarp, 584.
Scilla nonscripta, 108-10, Fig. 30, 111.
 scion, 133.
 sclerenchyma, 310, 313, 322-5, Fig. 77, 328, 339-42, Figs. 89-90, 372, 374-6.
 scorpioid, 575.
 Scrophulariaceæ, 435.
 scutellum, 37, Fig. 10.
 sea-kale, 88.
 sea lavender, 64.
 seaweed, 508-13.
 secondary, ray, 307.
 — thickening, root, 403-8.
 — — shoot, 119-21, 327-31, 348-57.
 — wall, 304, 314.
 — xylem, 308, 313-17, 329.
 secreting, cell, 442.
 — hair, 446, 447.
 secretory canal, 298.
 sedge, 172.
Sedum, 78.
 seed, 45, 74, 535, 538, 554.
 — dispersal, 560, 584-93.
 — structure and germination, 7-14, 28-41.
 — testing, 42-4.
 — viability, 44-8.
 seedless, 383, 583.
 seedling, 11, 110.
 segregate, 550, 553.
Selaginella, 526, 536.
 — *Kraussiana*, 526-9, Fig. 149.
 selected, 560.
 self-fertilisation, and selfing, 547, 550.
 self-pollination, 560, 562, 569, 579, 581.
 semi-parasite, 439.
 semi-permeability, 186, 258, 272, 419, 421.
 semi-permeable membrane, 17-19, 412.
Sempervivum tectorum, 78.
Senecio vulgaris, 59-63, 70.
 sepal, 469, 537.
 septicidal, 590.
 septifragal, 590.
 septum, 590.
Sequoia gigantea, 130.
 — *sempervirens*, 130.
 serrations, 159.
 sessile, 574.
 seta, 518-19.
 sex, 506-7.
 sheath, 89, 90.
 sheep's sorrel, 88, 403, Fig. 132.
 shepherd's purse (*Capsella*).
 shive, 365.
 shoot, 12, 14, 20, 50, 58, 64, 77.
 — apex, 51, 196-206, Figs. 52-4, 367-9, 401, 416, 473, 495-6.
 — direction of growth, 379-83.
 short shoot, 126.
 shrub, 115, 129, 130.
 sieve, plate, 303, 317-22, 355-7, 399, 405.
 tube, 317-22, Figs. 79-81, 339, Fig. 89, 355-7, Fig. 95, 376, 399, 411.
Silene inflata, 590.
 — *nutans*, 576.
 silicon, 425.
 silicula, 593, Fig. 169, 605.
 siliqua, 593, Fig. 169, 605.
 Silurian, 555.
 silver grain, 343.
 simple leaf, 66, 159.
Sinapis alba, 380, 386, 428.
 sleeping sickness, 455.
 sliding growth, 204, 338.
 slime strand, or string, 321.
 slipping, bark, 122.
 smear, 474.
 "snag," 123.
 snowberry, 179-83, Fig. 48.
 sodium, 420, 425, 426.
 — carbonate, 260.
 — nitrate, 430.
 — picrate, 272.
 softwood, 116, 121, 135, 342-3, 345-6.
 soil, 426-31.
 — particle, 411, 429.
 — solution, 411, 421.
 sol, 542.
Solanum, *Lycopersicum*, 425, 593.
 — *tuberosum*, leaf, 64, 161.
 — — nutrition, 427.
 — — oxidase in, 273-4; 278.
 — — starch in tuber, 243-4, Fig. 65.
 — — tuber and clone, 75, 83-7, Figs. 20-1.
 — — water loss from tuber, 284.
 Solomon's seal, 97, Fig. 23.
 solute, 18, 419-21.
 solution, 26.
 solvent, 18.
Sorbus Aucuparia, 147.

- sorrel, 161.
 sorus, 520, Fig. 147.
 South Africa, 113, 431.
 species, 3, 449, 455, 546, 551, 552, 554, 555, 556, 575, 595-7, 599-600.
 spectrum, 262.
 speedwell, field, 81.
 sperm, 510-13, Fig. 142, 515, 516, 523-5, 527, 529, 535, 537.
 spermogonium, 450, 454-5.
 spermatium, 450, 454-5.
 sphaeraphides, 424.
 spiderwort (*Tradescantia*).
 spike, 574, 575.
 spikelet, 562.
 spindle, fibre, 543.
 — nuclear, 542, 552.
 — threads, 542.
 spine, 65, 163, 164.
 spiral, arrangement of floral parts, 557, 602.
 — thickening, 305, 370, 372, 399, 414.
Spirogyra, 191-3, Fig. 51, 504-7, Fig. 140, 541.
 spongy mesophyll, 214, 219.
 spontaneous generation, 462.
 sporangium, 458, 460, 520-2, 537.
 spore, 5, 6, 450-5, 458-60, 467, 489, 523, 525-6.
 — mother cell, 522, 535, 545.
 sporogenous, 473, 474.
 sporophore, 457.
 sporophyll (see also megasporophyll and microsporophyll), 529.
 sporophyte, 519, 525, 528, 529, 535, 536, 538.
 spring wood, 120.
 spruce, 66.
 spur, 569, 580.
 spurious, 484, 583, 587.
 squirting cucumber, 585.
 stage, microscope, 175.
 stag-headed, 129.
 stalk, cell of antheridium, 535.
 — flower (see pedicel).
 — leaf (see petiole).
 stamen, 471-81, Figs. 124-5, 541, 557, 559, 562, 565, 572, 595, 605.
 staminate, 530-1, Fig. 150, 593.
 standard, 559.
 starch, 24, 36, 57, 208, 223, 226, 229-30, 239-47, 358, 465, 466, 473, 476.
 — grain, 243-6.
 — sheath, 296, Fig. 71.
 stele, 399-401, Fig. 110, 411, 412.
Stellaria, 573.
 — *Holostea*, 558, Fig. 156.
 — *media*, 70, 384, 480.
 stem, 51, 58, 64.
 — structure, 292-378.
 stereo-isomer, 237.
 sterigma, Fig. 120, 454.
 sterile floret, 575.
 sterilisation, 43, 270, 461-4.
 — partial, 428.
 stigma, 471, 480-1, 537, 562, 604.
 stimulus, 381, 443.
 stipe, 508.
 stipular scale, 148.
 stipule, 53, 147, 161, 605.
 stitchwort (*Stellaria*).
 St. John's-wort, 590.
 stock, animal, 272, 431.
 — grafting, 133.
 stolon, 81-3, Figs. 18-20, 94.
 stoma, 219-24, Figs. 62-3, 284-6, 290-1, 370-2, Fig. 100, 415.
 stone pine, 535.
 stonecrop, 78.
 stool shoot, 129.
 strain, + or -, 455, 460.
 Strasburger, 548.
 strawberry (*Fragaria*).
 streaming of protoplasm, 243.
Streptocarpus polyanthus, 67.
 striking a cutting, 133.
 style, 35, 39, 471, 481, 485, 537, 586, 587.
 sub-arctic, 79, 346.
 suberin, 361, 406, 428.
 submerged leaf, 165.
 succulent, fruit, 585.
 — plant, 164.
 sucker, 129.
 — of parasite (see haustorium).
 suction pressure, 411, 415, 417.
 Sudan III, 216, 251, 406.
 sugar, 24, 57, 224, 226, 569.
 sulphate, 265, 420.
 sulphur, 257, 423.
 sulphuric acid, 181, 217.
 summer wood, 120, 338.
 sundew (*Drasera*), 441-3.
 sunflower (*Helianthus*), 281.
 superior, 487, 557.
 survival, 560, 575.
 uspensor, 495-7, Fig. 137, Fig. 153, 536.
 utural, 484.
 swede, 71.
 sycamore (*Acer*).
 symbiosis, 432.
 sympetalous, sympetaly (gamopetalous), 559, 569, 572, 580.
Symphoricarpus albus, 179-83, Fig. 48.
Symphytum officinale, 559, Fig. 156, 569.
 symplastic, 205.
 sympodial, 124, 128.
 syncarpous, 471, 484, 559.
 synergidae, 490-1, 536, 537.
 synthesis, 239, 253.
 synthetic bundle, 312.
 syringa, 123, 130.
Syringa vulgaris, bud scale, Fig. 38, 146.
 — — habit, 115, 123, 127.

- TALIPOT palm, 72, 93.
Tamus communis, 159.
 tannin, 275.
 tap root, 93, 387-8.
 tapetal cell, 489, Fig. 133.
 tapetum, Fig. 124, 473, 474, 476, 522.
Taraxacum officinale, 88, 574, 587, Fig. 165.
 taxonomic, 2.
 taxonomist, 594, 607.
 tea, 135.
 teleological, 143.
 teleutospore, 453-4, Fig. 120.
 telophase, 543.
 temperature, 43, 149, 270.
 tendril, 446.
 tension (in tracheæ), 413, 414, 416, 417, 522.
 tentacle, 441-3.
 ternate, 159.
 testa, 9, 17, 44, 498, 591.
 tetrad, 478, 489, 519, 522, 527, 531.
 tetradynamous, 604.
 tetrarch, 403.
Thalictrum, 601.
 thallus, 509.
Theobroma Cacao, 120.
 thistle, field, 82.
 thorn, 64-5, Fig. 15.
 thrips, 581.
 thrum-eyed, 580-1.
 thyloses, 344.
Tilia vulgaris, 115, 123.
 — — bud scale, 147, Fig. 42.
 — — hairs, 290.
 — — phloem, 356, Fig. 95.
 — — root structure, 403-5, Fig. 111, 407.
 — — wood, 335, 336-8, Figs. 88-90.
 timber, 342-6.
 Timiriazeff, 263.
 tissue, 203.
 toadflax, 569.
 toadstool, 432, 454.
 tobacco flower, 576.
Tolmiea Menziesii, 66.
 tomato, 425, 593.
 tongue-grafting, 133, Fig. 35.
 toothwort, 243, 436-7, Fig. 116.
Tozzia, 436.
 tracheal elements, system, 411-13, 415-417, 421.
 tracheid, 316, 335, 343, 344-5, 375, 435, 442.
Tradescantia fluminensis, 90, 367, Fig. 99, 388, 472, 541.
 transitional, foliar, 118, 147.
 transpiration, 280-9, 413, 415.
 tree, 115, 129-31, 135-41.
 — — pollination, 562.
 — — radial growth of, 348-61.
 — — rise of sap in, 413-17.
 triarch, 399.
Trifolium pratense, 569.
 triple fusion, 492.
Trollius, 601.
Tropeolum majus, 52, 161, 287.
 tropical, and tropics, 135, 149, 151, 388, 431, 446, 561, 572.
 tropism, 379.
 true fruit, 583.
 trunk, 130, 135, 350, 418.
 tryptase, 253.
 Tschermak, 550.
 tube nucleus, 478, 479, 533, 537.
 — (see pollen tube).
 tuber, 83, Fig. 20, 241, 273, 284, 365, 439.
Tulipa, 105, Fig. 29, 111, 469-72, Fig. 123, 474, 476, Fig. 126, 470, 478, 492, Fig. 135.
 tunica, 204.
 tunicated bulb, 112.
 turgor, 223, 289, 411.
 turnip, 71, 193.
Tussilago Farfara, 82, Figs. 18-19, 456.
 tussocks, 93.
 tyrosin, 256.
Ulex europæus, flower, 559, Fig. 156.
 — — seed, 31.
 — — spine, 64, 65, Fig. 15, 163, Fig. 44.
Ulmus, 122, 126, Fig. 165, 356, Fig. 95, 586.
 — *procera*, 115, 123.
 — *glabra*, 115, 123-4, Fig. 32, 126.
Ulothrix, 502-4, Fig. 139.
 umbel, 574, 575.
 Umbelliferae, 574, 575, 584.
 unicellular plant, 190, 538.
 union, branch, 136.
 — graft, 134.
 unsaturated linkage, 251.
 uredospore, 452, Fig. 120.
Urtica dioica, 259.
Utricularia, 443-6, Fig. 118.
 VACUOLATED cell, 208-10, 495.
 vacuolating meristem, Figs. 53-4, 206-8.
 vacuolating and dividing cell, 206-8, Fig. 54, 330, 338, 495.
 vacuolation, 211, 474.
 vacuole, 184, 198, 415, 419.
 valency, 232.
 valve, 444, 590.
 van Bencden, 546.
 van Helmont, 225, 226.
 variegated, 258.
 variety, 75, 86.
 varnish, 251.
 vascular, 5, 520-38.
 — bundle, 293-6, 301-22, 374-6, 442, 444, 473.
 vaseline, 288-9.
 vegetative, cell, 533.
 — characters, 598-601, 605.
 — nucleus, 478.

- vegetative phase 58, 163.
 — propagation, 74, 78-88, 94, 105, 110, 131-5, 366, 383, 539, 554, 563.
 — shoot, 58, 64-7.
 Veihmeyer, 282.
 vein, 154, 155, 209, 211, 219, 292-3, 373.
 — ending, 158, 159.
 — islet, 158, 211.
 — systems, 158-60, 369.
 — water in, 286-7.
 velamen, 409.
 venter, 515, 523.
 ventral canal cell, 515.
 Venus' fly-trap, 443.
Veronica agrestis, 81.
 — *Chamedrys*, Fig. 105.
 — *serpyllifolia*, 493, Fig. 136.
 versatile, 477, 562.
 vessel, 306, 308, 314-17, Fig. 77, 333-5, Fig. 86, 405, 413-15.
 — differentiation, 351-5.
 — length, 352, 414.
 viability, 44-8.
Viburnum Lantana, 146, 150.
 — *Opulus*, 127.
Vicia Faba, boron, 425.
 — — in dark, 55, Fig. 14.
 — — nodule, 427.
 — — root, 410.
 — — seed, 3, 8-12, Figs. 1-3.
Vinca minor, 325.
Viola, 569.
 — *odorata*, 80, 81.
 violet, 80, 81.
 virtual image, 175.
 virus, 86, 448.
Viscum album, 439.
 vitamin, 279.
 vivipary, 46.
 von Frisch, 576.
 von Höhnel, 281.
 von Mohl, 183.
- WALL (see cell).
 wallflower (*Cheiranthus*).
 water, arrowhead, 160, 165.
 — balance in plant, 415-17.
 — buttercup, 165.
 — composition, 232.
 — content of, bud, 125, 151.
 — — — seed, 18, 45, 46.
 — — — wood, 351-5.
 — culture, 422-6.
 — dropwort, 87.
 — entry into, root, 410-15, 417.
 — — — seed, 15-19.
 — loss, 280-91.
 — movement across ring, 359.
 — plantain, 39, Fig. 13, 160-5, Fig. 45.
 — pollen transfer by, 561.
 — shoot, 129.
 — starwort, 165.
- water supply to tissue, 224.
 — vapour in tracheæ, 414.
 wax, 251.
 waxy scale, 446.
 wayfaring tree, 146, 150.
 weed, 42, 70, 426.
 Wellingtonia, 130.
 whip grafting, 134, Fig. 35.
 white deadnettle, 572-3, Fig. 161.
 whorl, 172, 470, 557.
 wild arum, 576.
 willow (*Salix*).
 willow herb, 591.
 — — rose bay, 68.
 wilting, 289, 415.
 wind, fruit dispersal, 586.
 — pollination, 529, 531, 561-5, 568.
 wing, fruit, 586.
 — pollen grain, 533.
 — seed, 535, 591.
 winter, 149, 417.
 withies, 133.
 woad, 605.
 wood (see also xylem), 115, 120, 439.
 — differentiation, 348, 352-5, 417.
 — diffuse porous, 338, Fig. 87.
 — fibre, 316, Fig. 77, 331-3, Fig. 85, 336.
 — parenchyma, 316, Fig. 333, Fig. 77, 86, 334.
 — ring, 119.
 — — porous, 336, Fig. 87, 343, 345, 352, 414.
 — structure, 331-46.
 woody perennial, 143, 386-90.
 wound, 274, 365.
 wrack, 508-13.
 wych elm, 115, 123-4, Fig. 32, 126
- XANTHOPHYLL, 259-61.
 xanthoproteic, 256.
 xeromorphic, 164.
 xeromorphy, 289-91, 372.
 X-ray, 245, 250.
 xylem (see also wood), 437, 440, 520.
 xylol, 195.
- YEAST, 267, 277, 278, 463-7.
 yellow rattle, 435-6, 591.
- Zeä mays, grain, 35-9, Figs. 10-11, 481.
 — — internode structure, 374-7, Figs. 102-4.
 — — leaf, 89-90.
 — — leaf structure, 369-74, Fig. 101.
 — — shoot apex, 367, Fig. 99.
 zinc chloride, 181.
 zoospore, 501, 502-3.
 zygomorphic, 573, 575, 580.
 zygosporic, 459, 460, 505-7.
 zygote, 501, 504, 539, 549.
 zymase, 465.

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