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PRACTICAL PLANT PHYSIOLOGY

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PRACTICAL PLANT PHYSIOLOGY

AN INTRODUCTION TO ORIGINAL RESEARCH FOR STUDENTS AND TEACHERS OF NATURAL SCIENCE MEDICINE AGRICULTURE AND FORESTRY

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By Dr. W. DETMER
Professor of Botany in the University of Jena

TRANSLATED FROM THE SECOND GERMAN EDITION

By S. A. MOOR, M.A. (Camb.), F.L.S
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WITH ONE HUNDRED AND EIGHTY-FOUR ILLUSTRATIONS

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PREFACE TO THE ENGLISH EDITION

THE teaching of Plant Physiology in England, which owes so much to the energy and enthusiasm of Francis Darwin, has been seriously retarded by the want of suitable manuals of laboratory practice, and hence no apology is needed for the appearance of a translation of Professor Detmer's well-known and excellent *Praktikum*.

No sufficient reason has been found for addition or alteration, and the Second German Edition has been presented in its entirety.

S. A. MOOR.

GONDAL, *March*, 1898.

AUTHOR'S PREFACE TO THE FIRST EDITION

It is by no means sufficient for the student of plant physiology to attend lectures, or wade through text-books on the subject. He must endeavour to familiarise himself, from practical experience, with the methods of research.

Plant physiology is now of such far-reaching significance for students of Natural Science, Agriculture, Forestry, and Medicine, that it has become a matter of importance to devote greater attention to it than hitherto in Universities and other advanced educational institutions. Especially it seems desirable to organise physiological exercises for students, and having myself, with very favourable results, introduced such a course of practical work in the University at Jena, I know from experience that the difficulties of the undertaking, which at first sight appear considerable, can be satisfactorily overcome.

In this book I have set before myself the task of advancing to the best of my ability the study of Plant Physiology. The *Practical Plant Physiology*, however, is by no means exclusively intended for the use of students. I trust that it may be welcome also to many teachers in higher grade schools. For many reasons Botany forms a particularly suitable subject of study for schools, and a series of physiological experiments not only gives a very special interest to the teaching of Botany, but also enhances its importance as a means of mental development.

The arrangement of the material in the book is essentially the same as that followed in my *Lehrbuch der Pflanzenphysiologie*, published in 1883 by E. Trewendt, Breslau. Theoretical considerations, to which in that treatise a considerable space was naturally assigned, are here almost entirely wanting.

My work during the last four years in the preparation of this *Practical Plant Physiology* has been very great. It has been

necessary to undertake a large number of the most various physiological experiments and microscopical observations, in order as far as possible to arrive at an independent judgment as to the value and practicability of the methods. Throughout the book special stress has been laid on the establishment of the relations between the anatomical structure and the physiological function of plant organs, and at the same time biological relationships have not been left out of consideration.

I have taken pains to make the apparatus suggested for the experiments as simple as possible, so that it can be put together by any one without great difficulty. At the same time, of course, certain complicated, and hence also expensive, instruments are indispensable, as, for example, a good microscope, a chemical balance, a spectroscope, an induction coil, a clinostat, etc.

W. DETMER.

JENA, *the end of September*, 1887.

AUTHOR'S PREFACE TO THE SECOND EDITION

IN presenting this Second Edition of my *Practical Plant Physiology*, I am more conscious than ever of the difficulties associated with the preparation of an introduction to physiological experiments. These difficulties are partly inherent, and partly due to the fact that the requirements of a course of practical work are by no means the same from all points of view.

The arrangement of the material is essentially the same as in the First Edition. In other respects, however, the book is essentially a new one, since almost every section has been enlarged or remodelled. I have included many new experiments for lecture demonstration or private work, and have taken pains to render the book increasingly useful to those who propose to make a serious study of plant physiology, and desire especially to familiarise themselves with the methods of research. Hence the efficiency of methods, in this Edition for the same reason often

described in considerable detail, has been critically examined with reference to concrete examples. It must, however, be expressly remarked that for the most part only methods of general significance could receive consideration, while methods worked out for special purposes have at most been briefly referred to, especially when satisfactorily dealt with in easily accessible original treatises.

Many new drawings, an index, and the appendix indicating where materials and apparatus may be obtained, will add to the value of the book.

I have taken great care in the selection of the research material recommended for the experiments. It will be found that not only material available in summer has received attention, but also material which may suitably be employed in winter. It is precisely in the less favourable season of the year that the proper choice of material is most important.

The number of experiments which I have made during the last four years to test methods of research has been very large I have also devised many new pieces of apparatus, which are described and figured in this edition.

It must further be remarked that the literature appearing after the middle of 1894 could not receive attention.

A French translation of the First Edition of my book, by Dr. Micheels of Ypres, was published at Paris in 1890.

My best thanks are due to the publisher for the great care he has taken in the get-up of the book.

W. DETMER.

JENA, *April*, 1895.

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FIRST PART.

Physiology of Nutrition.

FIRST SECTION.

The Food of Plants.

I. ASSIMILATION.

1. Proof of the Fact that Green Plants are Able to Produce Organic Substance from Inorganic Material.

THE fact that green plants can produce organic, *i.e.* carbonaceous, combustible substance from inorganic material, is of such fundamental significance, and the experiments to be made in order to establish it are so instructive, that very special attention must be paid to it. The investigations can be made at almost any time of the year; they certainly give by far the best results in summer owing to the favourable conditions for vegetation then prevailing, and if we are intending experiments in which the plants are to be brought to full development and seed ripening, it is clear that winter will not answer our purpose. For research material we may employ maize, wheat, oats, buckwheat, or beans.

It is first necessary to ascertain the dry weight of the dormant fruits or seeds used, in order to determine the quantity of organic substance contained in them. A few fruits or seeds are ground to a fine powder in a small hand-mill, and a small quantity of this powder, whose weight however must be accurately ascertained, will serve for the determination of the amount of dry substance contained in the originally air-dry material. About 3 gr. of the powder is placed in a suitable glass (weighing glass), and freed from water at 100° C. in the drying chamber. It is found that the air-dry fruits or seeds contain about 85 per cent. of dry sub-

stance. This last, it is true, does not consist entirely of organic bodies. There are present in it in addition mineral constituents, but the quantity of these is relatively so trifling, that we may here leave them out of consideration.

For the culture experiments which are to be made, we select a few fruits or seeds as perfectly developed as possible. Each of these is separately weighed and the weight noted. The previously made dry-substance determination now enables us to calculate the dry weight of each individual fruit or each individual seed. The objects are next placed separately in small glass or porcelain dishes, covered with water, and left in this for twelve to twenty-four hours to soak. They are then transferred to moist sawdust, contained in a suitable box or flower-pot, to germinate. It may here be remarked once for all that the sawdust, well soaked, must be rubbed between the hands, and placed in the vessels so as to form as loose as possible a seed bed. We lay the Phaseolus seeds horizontally in the sawdust, so that the emergent root forms a right angle with the long axis of the seed. Maize grains are so laid that the emerging root can grow straight downwards without making curvatures. So also we lay seeds of, *e.g.*, Vicia Faba in the sawdust with the micropyle directed downwards. When their radicles have reached a length of several centimetres the seedlings are cautiously removed from the sawdust, carefully washed, and further developed by the method of water culture.

For this we need in the first place suitable glass cylinders, which, if we are experimenting, *e.g.*, with maize plants, must be capable of holding about 2 litres of fluid. For smaller plants smaller vessels will suffice. The cylinders are filled not with pure water but with a food solution, in order to satisfy the requirements of the plants as regards mineral substances, a subject to which we shall return in detail later. The food solution may be prepared at once in large quantities, and kept in the dark in well closed vessels. A suitable food solution is obtained by using the specified quantities of the substances named below to 1 litre of distilled water.*

1·0 gr. Calcium nitrate ($Ca(NO_3)_2$);
0·25 gr. Potassium chloride (KCl);
0·25 gr. Magnesium sulphate ($Mg\,SO_4$);
0·25 gr. Potassium phosphate ($KH_2\,PO_4$).

* It is quite sufficient to weigh out the salts by means of a small pair of hand scales with horn scale-pans.

To the aqueous solution of these substances we further add a few drops of Ferric chloride solution.

I have often used this food solution with much success. A very satisfactory food solution is also obtained by dissolving the following in 1 litre of water:—

1·0 gr. Potassium nitrate;
0·5 gr. Sodium chloride;
0·5 gr. Calcium sulphate;
0·5 gr. Magnesium sulphate;
0·5 gr. tribasic Calcium phosphate.

The Calcium phosphate is very finely powdered before use. It is very sparingly soluble in water, and consequently forms a sediment at the bottom of the culture vessel. The food solution is completed by addition of a few drops of Ferric chloride solution.

Having filled the cylinders with food solution, and selected for each a suitable cork provided with a large hole, the seedlings are fixed in the holes of the corks by means of cotton wool (see Fig. 1). Each plant requires a separate cylinder. The roots must extend into the food solution; the still present receptacles of reserve material (endosperm or cotyledons) should not dip into it, but still must not be allowed to dry up. The culture vessels with their plants are placed in front of a window, where the latter are exposed to direct sunlight. Over the cylinders however is pasted black glazed paper, so as to prevent the development of algæ in the food solution and on the roots of the plants. The white side of the paper must be outside, and then the fluid in the culture vessels does not get too warm. A still more simple plan for screening the roots from access of light is to place each culture vessel in a cardboard cylinder. It is obvious that as vegetation proceeds care must be taken to replace frequently by distilled water the water absorbed from the food solutions.

The precautions to which attention must be paid when it is desired to bring plants to an advanced stage of development by the water-culture method, will be discussed later in this section. We are at first only concerned in proving that green plants in general can produce organic substance, and it is therefore quite sufficient if they vegetate for a few weeks, and produce vigorous stems, leaves and roots. We then remove the plants from the food solutions,

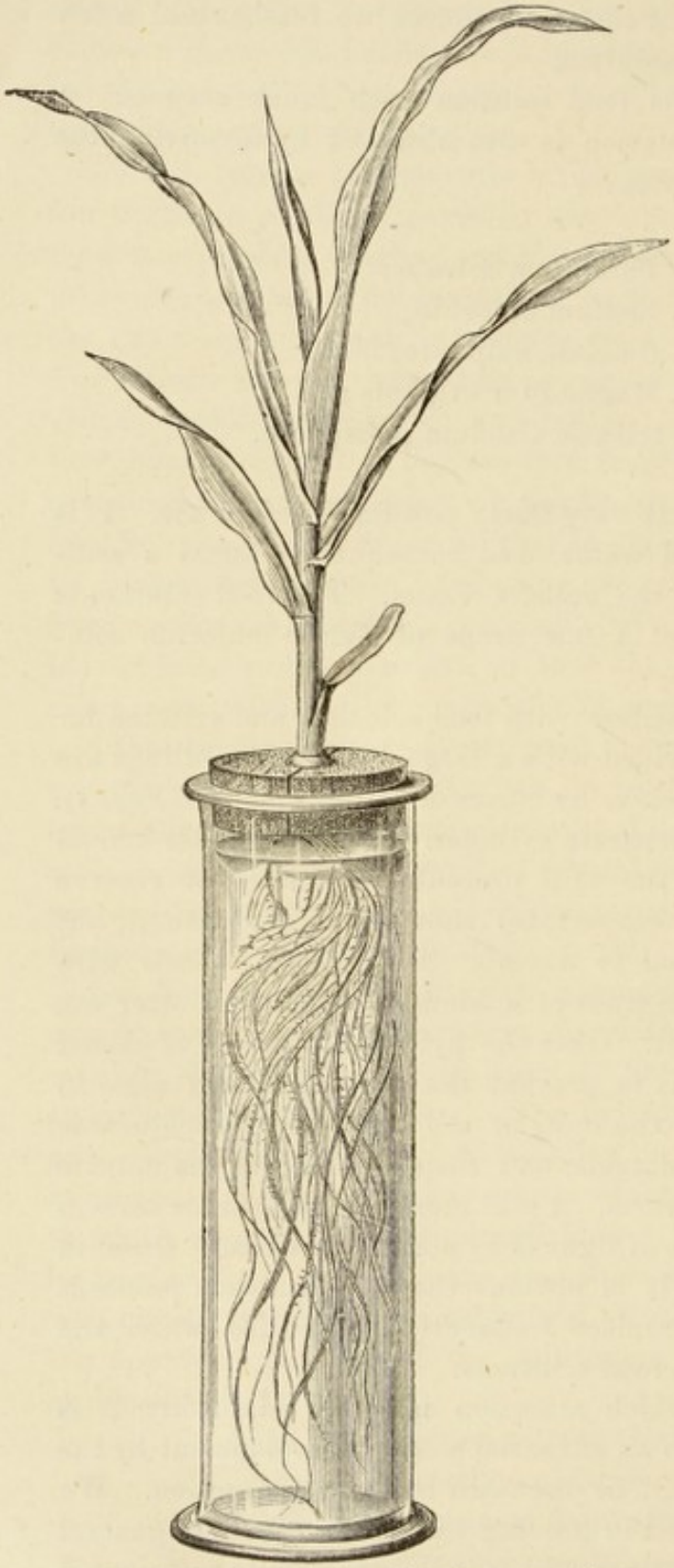

FIG. 1.—Maize plant, developed by the water-culture method.

reduce them as quickly as possible to the air-dry condition by exposure to the air, then with scissors cut up each plant separately as finely as possible, and use either the whole mass of an individual or a weighed portion of the air-dry substance for the determination of the dry weight.

If we compare the dry weight of a plant with that of the seed, we find that the former is many times greater than the latter. Since the ash of the plants obtained, like that of the seed, is relatively very small in quantity, it follows that a large quantity of organic substance has been produced by our plants. We have however placed at their disposal no organic substances, but merely water, some salts, and the constituents of the air. And our cultures therefore indicate that the research objects used are able to produce organic bodies from purely inorganic material.

If it is proposed to make extended water-culture investigations,

and bring the research objects (oats, maize, buckwheat, etc.) to complete development and fruit ripening, the cultures will naturally require far greater attention, than when the object is merely to prove that green plants in general are able to produce organic substance.

1. The seeds or fruits are soaked for twelve to twenty-four hours in distilled water, and then germinated in well-washed sawdust loosely filled into flower-pots. (Further particulars have already been given above.) Under some circumstances, and especially in accurate investigations of the requirements of plants as regards mineral substances, it is advisable to germinate the seeds or fruits, after soaking, between folds of Swedish blotting-paper. After soaking the seeds are placed horizontally or vertically between many times folded blotting-paper, which is then fixed upright with its lower end dipping into distilled water, which covers the bottom of a wide-necked glass vessel. The arrangement is finally covered with a bell-glass, the tubulure of which is loosely plugged with cotton wool.

2. When the roots of the seedlings have attained a length of a few cm., they are transferred to culture vessels of 1–4 litres capacity according to circumstances. The culture cylinders can be closed by means of a cover provided with three openings, and made of japanned sheet zinc, or better of porcelain. The middle opening is fitted with a halved and perforated cork. In this perforation a seedling is fixed as above described by means of cotton wool. Or we may fix the seedling directly into the opening (about 3 cm. in diameter) of the cover without using a cork. The second opening of the cover is closed with a perforated cork, which holds firmly the lower end of a thin wooden rod intended to support the plant during its development. The third opening of the cover is closed with a non-perforated cork.

3. The shading of the roots in the culture vessels is easily attained by putting these into suitable cardboard cylinders, or by wrapping the cylinders with several layers of flannel. Care must be taken on hot sunny summer days that the food solution does not get too warm.

4. The culture vessels are placed at the window of a room with a south aspect. It greatly promotes the development of the plants if the culture vessels are placed as often as possible in the open air in front of the window. If we make extended researches every year with the help of the water-culture method, it is very

desirable to set up a special plant house, made of glass and iron, and so constructed that the culture vessels standing on small trollies can readily be brought into the open as often as possible.[1] *

5. Obviously care must be taken to replace frequently the water lost by the food solutions. When the plants have formed a vigorous root system, it is advisable to remove them, for one to two days, say, every week, from the food solutions, and transfer them to distilled water.

6. Great care must be taken to keep the food solution slightly acid in reaction. An alkaline reaction, which is highly injurious, may be remedied by addition of a little Phosphoric acid.

7. To keep the roots in the food solution adequately supplied with Oxygen, and prevent the formation of Ferric sulphide which may take place with deficiency of Oxygen, it is advisable to pass a stream of air through the food solution once or twice every day. This is most simply done by means of a gasholder. The glass tube conducting the air passes to the bottom of the culture vessel through the third opening above mentioned of the cover.[2]

8. Very frequent renewal of the food solution is by no means necessary, and not even of advantage, as experience has frequently shown. As a rule it is sufficient, if we are working with culture vessels of about 3 litres capacity, to renew the solution two or three times in the course of the summer. It is convenient to prepare beforehand concentrated solutions of the nutrient salts, and then dilute them to the proper strength for use. For example, we may dissolve in every 50 c.c. of water, 1 gr. Calcium nitrate, 0·25 gr. Potassium chloride, and 0·25 gr., acid Potassium phosphate, and in a second solution keep on hand 0·25 gr. Magnesium sulphate to every 50 c.c. of water. The food solution when made up will only require the addition of a few drops of Ferric chloride solution. It is advisable to remove the plants from the food solution when the stage of fruit ripening draws near, and to supply them with distilled water only, which, if the researches permit it, is very slightly acidified with Nitric or Phosphoric acid.

At the time of flowering care must be taken that normal fertilisation takes place. It is generally sufficient to let the plants

* It is advisable to enclose the space intended for the plants outside the plant house with wire netting, so as to keep away birds.

stand in the open air, *e.g.*, outside the window or behind the open window. The transference of the pollen to the stigma can then be effected by insects.

As we have said, considerable warming of the food solution, and also deficiency of Oxygen in it, may be very prejudicial to success in cultivating plants by the water-culture method. Wortmann[3] has recently described a simple method for considerably reducing, if not completely eliminating, these dangers, a method which is particularly to be recommended when it is desired to obtain handsome plants by the method of water culture for purposes of demonstration. We cultivate the plants, viz., in very large vessels. Suitable glasses are to be obtained at a price of 5 marks from Ehrhardt & Metzger in Darmstadt. They are 60 cm. high, 25 cm. in diameter, and hold 26½ litres of water. We fill the glass cylinders, if only demonstration experiments are intended, with spring water, introduce the germinated plants (*e.g.*, Phaseolus), and let them vegetate for six days. We then add to the water sufficient nutrient salt (its composition may, *e.g.*, be such as is specified on p. 3) to give a concentration of 1 per cent. After three to four days the strength is raised to 2 per cent., and after three to four days longer to 3 per cent. Every three or four days the food solution is thoroughly well stirred up. It need not be renewed, but care must be taken to replace the evaporated water. To shade the roots it is sufficient to wind round the cylinders one to two layers of white linen or woollen.

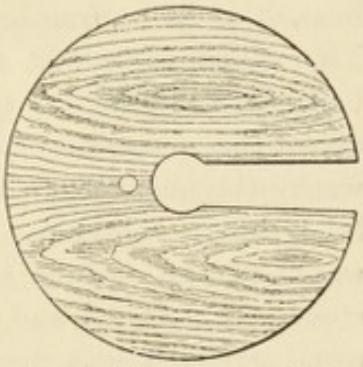

Fig. 2.—Wooden cover for a culture vessel.

For the cover of the culture cylinder we use a round piece of wood, which projects a little beyond the margin of the vessel. The cover is easily prevented from slipping about by driving in a few nails from below. In the middle of it is a hole 3 cm. in diameter, in which the plant can be fixed in the usual way. Just beside this hole is another, in which is fixed a not too thick rod, which serves to support, *e.g.*, a Phaseolus plant. From the central hole a slot about 2·5 cm. broad runs to the periphery of the cover. By means of this the research plant can be readily introduced or taken out. The slot is closed by means of the strip of wood which is removed in making it. The cover, seen from

above, is represented in Fig. 2. In a vessel arranged as above, which contained only about 5 litres of water, I brought a Phaseolus plant to splendid development. A complete renewal of the food solution was not made from the commencement of the experiment till the time of flowering, when the plant was above the height of a man. I often found that in my laboratory, where Maize grew vigorously, that beans did not develop normally. Relatively dry air and the products of combustion of gas appear to influence the growth of beans far more injuriously than, *e.g.*, that of the Maize; the former consequently must always, when a plant house is not available, be cultivated in the open air outside the window.

An excellent object for water cultures intended for purposes of demonstration is the willow. For example, early in February, 1894, I introduced into the cover of the 5 litre culture cylinder a branch of Salix fragilis, about 25 cm. long and 2 cm. in diameter. The vessel contained at first only spring water, in which the branch was immersed to half its length. Over the portion rising above the cover was placed a glass cylinder. At the end of four weeks the buds began to burst, and the roots had already become fairly long. The glass cylinder was now removed, and the spring water in the culture vessel was replaced by food solution (half strength). At the end of the next four weeks food solution of the usual strength was put into the culture vessel. Every day the evaporated water was replaced, and every two months the food solution was renewed. In the course of the summer a vigorous root system developed, and six long, branched, very woody, perfectly normal lateral branches with numerous leaves. During the summer the plant was often placed in the open air outside the window. At the beginning of September the food solution was replaced by spring water; the plant was kept in a warm room behind a window looking to the south. It cast its leaves quite gradually from the middle of October to the end of December, and now (January 10, 1895) is still in a state of winter dormancy.

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[1] Respecting suitable plant houses see Wolff, *Versuchsstationen*, Bd. 8, p. 485; and Nobbe, *idem*, Bd. 12, p. 477.

[2] Gasholders are to be obtained from R. Muencke, Berlin, Luisenstr. 58. The *Wasserstrahlgebläse*, also to be obtained from Muencke, are likewise very convenient for passing air; they can be attached in the simplest manner to the water supply. See Muencke's Catalogue, 1886, Pt. 1, p. 76.

[3] See Wortmann, *Botan. Zeitung*, 1892.

2. The Production of Organic Substance in Green Plant Cells under the Influence of Light.

The production of organic material in the green plant cell is dependent on the co-operation of light, an important doctrine in plant physiology, of the truth of which we must satisfy ourselves by experiment. We weigh separately a few maize grains, and estimate their dry weight. (See 1.) After soaking the grains and germinating them in sawdust, each seedling is separately fixed, in the manner described in 1, in a cylinder filled with food solution. Some of the culture vessels are now placed in darkness under a large cardboard box; the others, under otherwise similar conditions, are exposed in a very well lighted place to the alternation of day and night. The leaves of the plants kept in the dark do not become green like those of the illuminated plants, but assume a yellow colour, since the normal green chlorophyll pigment can only develop in the cells when they are exposed to the light. After four or five weeks we remove the plants from the culture solutions, dry them in the air, and determine the weight of dry substance in each individual, which weight is then to be compared with that of the corresponding seed grain. The dry weight of the illuminated plants is found to be considerably greater than that of the maize grains started with, while the dry weight of the plants grown in the dark, is, as I ascertained, about 50 per cent. less than that of the seeds. In absence of light no formation of new organic material can take place, but on the contrary a large portion of the organic bodies already present are broken down in metabolism (respiration). In presence of light it is true the breaking down of organic material in respiration also takes place, but the losses due to this are more than covered by assimilation, so that the dry weight of plants vegetating in the light progressively increases.*

3. The Organs of Assimilation.

If we consider in the first place only what is presented to us in the higher plants, it is to be noted that most of them have well developed foliage leaves. These are to be placed in the very first rank as organs of assimilation. Their blade presents a large

* For similar experiments see Detmer, *Versuchsstationen*, Bd. 14.

surface to the Carbon dioxide-containing air, and their green tissue is kept extended by the special arrangement of the nerves. The nerves also convey to the mesophyll the water and mineral substances necessary for the life and functions of the cells of the chlorophyll-containing parenchyma. The arrangement of the nerves in the leaf is of course very different in different plants, and we may proceed as follows to obtain special information on this subject. A leaf of Impatiens parviflora is laid in alcohol till the chlorophyll is extracted. We then place the leaf for a time in a solution of 5 parts of chloral hydrate in 2 parts of water. By this means the leaf is rendered highly transparent, and por-

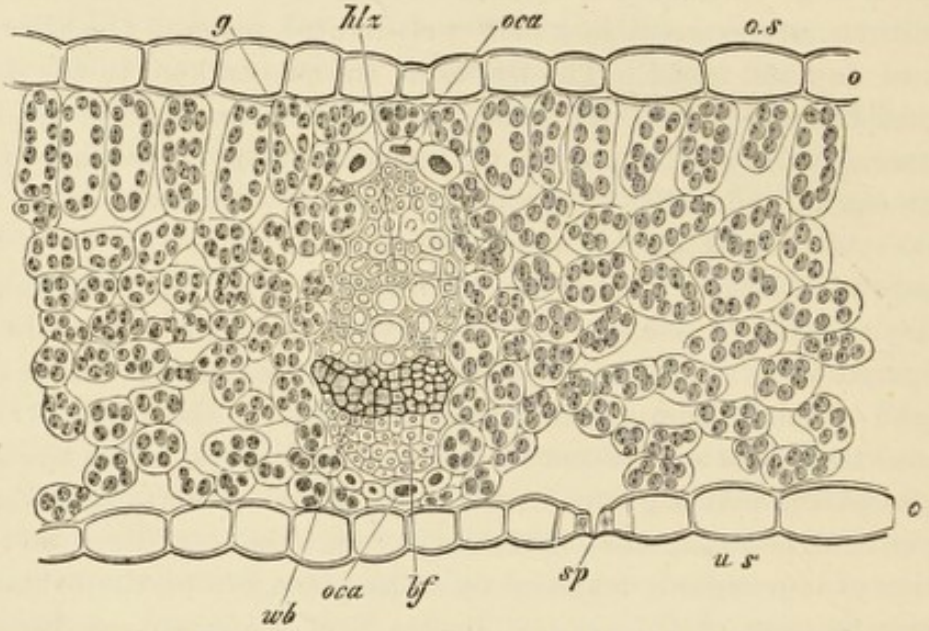

FIG. 3.—Transverse section of part of a mature leaf of Trifolium pratense: *o.s*, upper side, *u.s*, under side of the leaf; *o*, epidermis; *sp*, stoma; *oca*, crystal of Calcium oxalate in the crystal sheath of the vascular bundle; *hlz*, wood of the vascular bundle; *g*, vessels; *wb*, soft bast; *bf*, bast fibres. Magn. 300. (After H. de Vries.)

tions of it may be submitted at once to microscopic examination. In the mesophyll we perceive elongated cells, which contain bundles of raphides (crystals of Calcium oxalate). The leaf is traversed by a fairly strong median nerve, from which lateral nerves of the first order run to the edge of the leaf, where, curving upwards in an arc, they join on to the next higher lateral nerves of the first order. The nerves of the first order give off nerves of the second order, these nerves of the third order, and so on, so that a much divided network is formed, of which the ultimate very fine branches in part end blindly in the mesophyll.[1]

In order to gain general information as to the anatomical structure of the mesophyll, we now proceed to prepare as thin as possible transverse sections of leaves, and select for examination say the foliage leaves of Dahlia variabilis, Vitis vinifera, Berberis vulgaris, Syringa vulgaris, Trifolium pratense, Ilex, or Fagus sylvatica. On microscopic examination of the sections, it is at once evident that the green mesophyll is not of the same structure on the upper and lower sides of the leaf. (See Fig. 3.) Below the epidermis of the upper side of the leaf we find tubular cells, elongated at right angles to the surface, and termed palisade cells, while on the under side of the leaf is developed spongy parenchyma abounding in intercellular spaces. The cells both of the palisade and of the spongy parenchyma contain chlorophyll grains, but the former, for reasons which cannot be set forth in detail till later, is of special importance for vigorous assimilation, and hence its occurrence on the upper side of many leaves demands special attention.

But even facts of comparative anatomy are of importance in affording a basis for the view that the palisade parenchyma must be regarded as a specific assimilatory tissue.

Sarothamnus vulgaris is a shrub bearing only very small leaves, which appear inadequate for the work of assimilation. Here the much branched stem system must be operative as well as the leaves, in order that sufficient quantities of organic substance for the plant may be produced. A transverse section of the stem presents the appearance of a five-rayed star, and if we examine a thin section under the microscope, it will be found that under the epidermis of the ends of the rays sclerenchymatous tissue is present while the peripheral tissue of the intervening hollows is green. We determine that this green tissue in its outer layers consists of palisade cells, which are elongated at right angles to the surface of the stems, while further inwards follow more isodiametric chlorophyll-containing cells. We also examine a thin transverse section of the stem of Spartium junceum. The assimilatory tissue below the epidermis here consists entirely of palisade cells. There are about six layers of much elongated green cells arranged at right angles to the surface of the stem. The green tissue does not however form a closed ring below the skin, the assimilatory tissue alternating at the periphery throughout the entire stem with sclerenchyma. In plants with poor foliage, or plants which do not produce any green leaves at all, the green tissue of the stem system must undertake the work of

assimilation to a very large extent or altogether, and this tissue hence consists chiefly or entirely of palisade cells.

The possession of palisade parenchyma however is by no means characteristic of the foliage leaves of all plants. If, for example, we examine transverse sections of the young leaves of Triticum vulgare, we find that the mesophyll, which is bounded by an epidermis both on the upper and the lower surface of the leaf, and is traversed by vascular bundles, is composed throughout of nearly uniform cells, roundish in transverse section. Leaves in which differentiation into palisade and spongy parenchyma does not take place are frequently distinguished by the fact that not the whole of the tissue enclosed by the epidermis consists of chlorophyll-containing cells. If, *e.g.*, we examine a transverse section of a leaf of Iris germanica, we shall find green tissue below the epidermis of the upper and of the under side of the leaf. The vascular bundles stand out distinctly, the bast being covered on the outside by a layer of bast fibres, and we further see at once that the middle layer of the leaf consists of cells full of sap and not green. Such chlorophyll-free cells are also very abundant in the leaves of Hyacinthus orientalis and the succulent leaves of Aloes.

Reference must here also be made to the interesting fact that leaves with well developed spongy and palisade parenchyma appear for that very reason highly dorsiventral in construction, and rank among extremely plagiotropic organs. But by no means all foliage leaves are strongly dorsiventral in construction; there are many dicotyledons in which the mesophyll of the leaves is centric in organisation, and these for the most part exhibit a more orthotropic development. Thus, *e.g.*, the leaves of Anchusa italica, Centaurea Jacea, Tragopogon orientalis, Aster Amellus, Genista tinctoria, etc., are centric in structure. In the last the mesophyll consists almost exclusively of cells elongated at right angles to the surface of the leaf. Centaurea Jacea varies considerably, as I have often found, according to its habitat. Individuals well exposed to the sunlight produce long narrow leaves which are comparatively thick. The leaves of shaded plants are thinner, but greater in area. The mesophyll of the leaves of Centaurea Jacea, especially in plants exposed to the sunlight, is not dorsiventral but centric in structure. We easily see, on microscopic examination of delicate transverse sections, that palisade parenchyma is present both on the upper and under sides of the leaves.[2]

Leaf stalks are generally poor in chlorophyll-containing cells, since they do not for the most part function as organs of assimilation, being very differently occupied. Microscopic examination of a transverse section of the leaf stalk of Vitis vinifera teaches that close under the epidermis groups of collenchyma bundles are present, and that between these bundles occurs feebly developed green parenchyma together with cells, of which sometimes many, sometimes only few, contain a red-coloured pigment dissolved in the cell sap. The cortex of the leaf stalk, consisting of the various tissues mentioned, surrounds the ring of vascular bundles and the pith. Examination of the leaf stalks of other plants also, *e.g.*, those of Chenopodium bonus Henricus, shows them to be very poor in green tissue.

We further prepare a transverse section of leaf stalk of a Begonia (I examined especially Begonia manicata). Below the epidermis comes a ring of collenchyma, then large-celled ground tissue in which the bundles are not arranged in a circle. The peripheral layers of the ground tissue do indeed contain chlorophyll, but the chlorophyll grains, although relatively large, are only few in number.

The green stems of plants, like the leaf-stalks, usually participate only to a very limited extent in the work of assimilation, and hence the bulk of their tissue contains no chlorophyll grains. If, *e.g.*, we prepare a transverse section of a poppy stem, we observe in the centre the pith. Outside this come the vascular bundles, each of which possesses, in addition the wood, a broad zone of soft bast, and a bast fibre region outside this. The medullary rays between the individual vascular bundles are composed of large cells. In the cortex the presence of a closed cylinder of sclerenchyma is especially characteristic; this is surrounded on the outside by a layer of green tissue feebly developed in proportion to the mass of the stem, and immediately on this chlorophyll parenchyma adjoins the epidermis. We still further examine a transverse section of the stem of Chenopodium bonus Henricus, and find that under the epidermis collenchyma and green parenchyma alternate with one another.[3]

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[1] See Sachs, *Lectures on Plant Physiology*.

[2] See Heinricher, in Pringsheim's *Jahrbücher*, Bd. 15.

[3] Literature on assimilatory tissue: Pick, *Beiträge zur Kenntniss des assimilirenden Gewebes armlaubiger Pflanzen*, Bonn, 1881; G. Haberlandt, Pringsheim's *Jahrbücher*, Bd. 13; Stahl, *Botan. Zeitung*, 1880.

4. The Transparency of Plant Tissues.

Light is of great importance in connection with very many physiological processes in plants, and rays of unequal refrangibility must by no means be considered equivalent as regards the influence which they exert on plant life. It is therefore not without interest to make some experiments respecting the transparency of tissues. The depth to which rays of light penetrate into plant tissues depends on the one hand upon the intensity and the refrangibility of the light rays, and on the other upon the chemical character of the constituent parts of the cells themselves, and the anatomical structure of the tissues. As regards this last, the presence of a more or less extensive intercellular system *inter alia* plays an important part. If, *e.g.*, many intercellular spaces occur, the incident rays will be obliged to pass very frequently from the cell sap, and through the cell walls with their imbibed water, into air, and this naturally will greatly diminish the transparency of a tissue. The significance of the intercellular spaces in this respect will at once be manifest, if we make the following experiment. A piece of Begonia manicata leaf is laid in water, contained in a small glass. We close the mouth of the glass with a rubber stopper, through which has been passed one end of a glass tube bent at right angles, the other end of this tube being connected with an air pump. On exhausting, air escapes from the intercellular spaces; these however fill with water, and the leaf now appears far more transparent than at the commencement of the experiment. If we immerse the blade of a leaf of Primula sinensis in water, take the end of the leaf-stalk in the mouth, and remove air from the intercellular system by sucking, water penetrates into the intercellular spaces of the leaf through the stomata, and the leaf is thereby rendered fairly transparent.

Cork tissue, owing to the specific nature of its cell contents, is only slightly transparent. Similarly tissues rich in chlorophyll absorb much light owing to the presence of the pigment, and transmit only comparatively little. Here also it may be mentioned, although we shall consider the matter in more detail later, that the chlorophyll pigment has the power of absorbing very energetically the so-called chemical rays. We can easily prove this by laying any green leaf on a piece of photographic paper, and exposing between two sheets of glass to the influence of the light. The portion of the paper not covered by the leaf rapidly becomes

brown; the covered part loses its white colour only slightly, or not at all, since the chlorophyll pigment absorbs the so-called chemical rays very energetically.

To determine the depth to which light of an intensity still perceptible to the eye penetrates into the tissues, we use, after Sachs,[1] the simple diaphanoscope (Fig. 4). This consists of a tube *a*, 60 mm. long, and 35 mm. in diameter, made of stout cardboard. This tube is open at one end, but closed at the other, except for a small opening about 10 mm. in diameter. Over this end of the tube *a* slips an exactly similar cardboard tube *b*. If we place the object under investigation between the tubes, hold the open end of *a* close to the eye, directing the instrument towards the sun or a bright cloud, we can investigate the transparency of plant tissues. I inserted a piece of Lonicera tatarica leaf in the diaphanoscope; the light transmitted was bright green. Using four pieces of leaf, it was at once clearly seen that green light passed through. With six pieces, the eye perceived, after looking into the apparatus for some time, a yellowish glimmer.

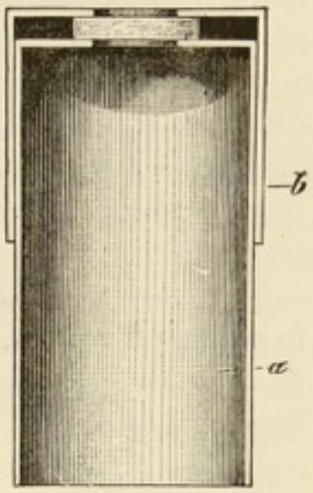

FIG. 4.—Diaphanoscope, in longitudinal section.

To construct an analysing diaphanoscope, it is only necessary to slide a simple diaphanoscope over the front end of the tube of a suitable spectroscope. During observation we direct it against bright clouds or the blue sky. I placed a piece of Syringa vulgaris leaf in the diaphanoscope, and found that it permitted the passage of red, orange, yellow, green, and some blue, though certainly diminished in intensity; the more refrangible rays were completely absorbed. Two pieces of Syringa leaf only transmitted red, orange, yellow, and green very much weakened. A slab of tissue 17 mm. thick from the parenchyma of a potato tuber absorbed the more refrangible rays completely, but transmitted much weakened red, orange, yellow, green, and a trace of blue. According to these observations, then, the less refrangible rays penetrate further into the plant tissues than those of high refrangibility.

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[1] Cf. Sachs, *Sitzungsb. d. Akad. d. Wiss zu Wien*, 1860, Bd. 43. See also Engelmann, *Botan. Zeitung*, 1887, p. 393.

5. The Chlorophyll Bodies.

The chlorophyll bodies are to be regarded as organs of assimilation. Their form is usually discoid; in the cells of many algæ chlorophyll bodies of other forms occur. We take for example a few threads of an alga which frequently occurs in stagnant waters, viz., a species of Zygnema, place them on the slide in a drop of water, cover with a cover-glass, and examine under a magnification of about 500 diameters. It is seen that each filament is made up of a row of cells, and that in each cell there are present two green, star-shaped structures, the chlorophyll bodies. We also see the cell-nucleus in the middle of each cell.

The various kinds of Spirogyra are algæ which mostly occur in stagnant waters, and consist of unbranched filaments of cells. In each cell we perceive on microscopic examination green spiral bands, varying in number in different kinds, which constitute the chlorophyll bodies. The parietal plasma and the nucleus suspended in the cell-sap by means of thin plasmic threads are often easy to observe. It may further be noted that at intervals in the bands of Spirogyra are embedded spherical colourless structures, the amylum bodies, which enclose a pyrenoid of angular contour composed of proteid substances. The pyrenoids are surrounded by numerous small starch grains.

Once we have found good Spirogyra material, we should attempt to cultivate it, since it is required for many physiological experiments. It is best to proceed according to the method of Strasburger. The algæ are transferred to vessels, not too deep, containing spring water. The walls of the vessels must either be non-transparent, or rendered opaque by pasting black paper over them. We expose the algæ to bright diffuse daylight (not to direct sunlight), and from time to time drop into the water small fragments of peat which have been boiled and then soaked in the ordinary food solution used in water culture experiments.

Species of Cladophora, algæ with branched filaments rough to the touch, are frequently present both in standing and running waters. The lateral branches spring from the upper end of the cells. Fairly high magnification is employed for further investigation, and it then appears that the green parietal layer of the cells is composed of small polygonal structures which are separated from one another by delicate colourless lines. The chloro-

phyll bodies of Cladophora are already much like those of higher plants.

Mention may also be made here of a remarkable organism which is met with in pools, clinging to the submerged parts of plants. I refer to Hydra viridis, a small water animalcule, 5–12 mm. in length, and green in colour. If we transfer the Hydra to a drop of water, and, without putting on a cover-glass for fear of injuring the creature, examine it under the microscope, we shall readily make out that it is a sac-like structure composed of two layers, the ectoderm and the endoderm, that at the anterior end it has a mouth opening (a posterior opening is wanting) surrounded by many tentacles, and that it can contract itself and then again stretch out. In the endoderm of the cylindrical body of the Hydra, and also in the endoderm of the tentacles, we observe numerous green globular structures. These are unicellular algæ which live in symbiotic relation with Hydra viridis. The Hydra affords protection to the algæ while they serve the Hydra by providing it, through their assimilatory activity, with organic substance and free Oxygen.

In plant houses in which ferns are cultivated it is generally easy to find on moist walls, or on the stems of tree ferns, fern prothallia, small green mostly heart-shaped structures, closely applied to the substratum. We remove some with the forceps, and after rinsing them with water, examine them microscopically in a drop of water. Except along the middle they are composed of a single layer of cells; they usually have a notch at their anterior end, and produce on their lower or ventral surface fairly long root hairs. For our present purpose it is specially important that in the green prothallium cells numerous chlorophyll bodies are readily seen.

It is further instructive to examine under the microscope a leaf of the widely distributed moss Funaria hygrometrica. For reasons which need not here be discussed, we select plants which have been exposed for some time to diffuse daylight, and find that the cells of the leaf, which up to the midrib is unilamellar, contain many large chlorophyll grains, some of which are undergoing division.

We further prepare a transverse section through the thallus of Marchantia polymorpha (a dichotomously branching liverwort, very frequently occurring on moist soil). Without going into details it is sufficient here for us to determine the presence on the

upper side of the thallus of a tissue rich in chlorophyll. Then comes a middle layer poor in chlorophyll, and on the ventral side we find again two layers of cells richer in chlorophyll.

If a leaf from a bud of Elodea canadensis is mounted on the slide in a drop of water and examined microscopically, we shall find after a short search cells which present the relations represented in Fig. 5. The parietal plasma, the nucleus-containing mass of protoplasm, and the protoplasmic threads proceeding from the latter, are easy to see. Frequently the protoplasm exhibits fairly active movements, and in the plasma the chlorophyll bodies stand out distinctly.

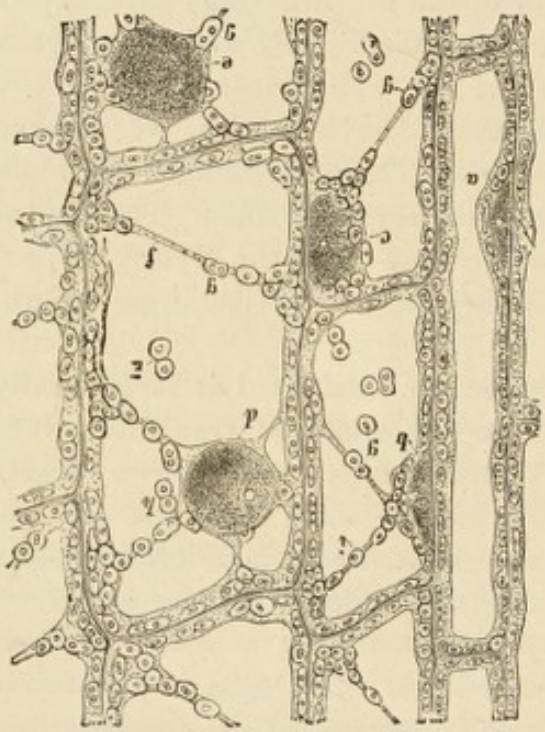

FIG. 5.—Cells from the leaf of Elodea canadensis: *a* to *e*, cell-nucleus; *f*, protoplasmic bands; *g*, chlorophyll grains, some in a state of division, and containing starch grains. (After Kny.)

If the outer layers of cells are removed from the lower side of an Echeveria leaf, and we examine sections of the loose tissue lying below, we shall perceive in the uninjured cells large chlorophyll grains, which are particularly interesting, inasmuch as, when examined under fairly high magnification, they exhibit comparatively well a foamy structure. We may assume that all chlorophyll grains possess such a structure; it is however by no means always easy to make out.

If Lupin seeds are germinated in the light, the cotyledons, appearing above ground, assume a green colour. Microscopic investigation of a transverse section of a cotyledon at once reveals the epidermis, the ground tissue, and the vascular bundles, and in the cells of the ground tissue, especially in the peripheral ones, are to be recognised many relatively large chlorophyll grains.[1]

It is of importance to emphasize here the fact that there are many plant structures which do not appear chlorophyll-green, but which still contain larger or small quantities of chlorophyll, and are therefore capable of assimilatory activity. We prepare

transverse sections of the leaves of red-foliaged varieties of Corylus or Fagus. In the cells of the palisade parenchyma and of the spongy parenchyma, as in the corresponding parts of green leaves, are present numerous chlorophyll grains, but the epidermal cells contain red or violet-coloured cell sap. In the uninjured leaves, therefore, the colour of the chlorophyll is merely masked by the pigment present in the epidermis. The young leaves of many plants (*e.g.* of oaks) are red, the leaves not becoming green till later. The mesophyll of the young leaves contains numerous chlorophyll bodies, as we may satisfy ourselves by studying transverse sections, but in the cell sap of the cells of the assimilatory tissue, especially of the palisade parenchyma, are dissolved red colouring matters. In this case the red pigment serves to protect the young, more deeply situated green cells against too intense light.

Neottia Nidus avis is an Orchidaceous plant which is frequently met with in the humous soil of damp woods. The whole plant is brown in colour; it appears to contain no chlorophyll, a transverse section of the stem from a place about 6 cm. below the inflorescence exhibits clearly on microscopical examination the epidermis, the parenchyma of the cortex and of the pith, and between these a cylinder of sclerenchyma, together with the vascular bundles; green chlorophyll bodies, however, are nowhere to be found. If a Neottia plant is crushed and treated with alcohol, it is easy to obtain a chlorophyll-green extract, which fluoresces also in a manner characteristic of a chlorophyll solution. In fact, Neottia, as Wiesner[2] first found, contains chlorophyll; the plant can therefore assimilate, and itself produce from inorganic material a portion of the organic substance which it requires, at the same time it is true obtaining another portion from without. For further information we make the following observation. We strip a fragment of epidermis from the ovary of a Neottia flower, and examine it under high magnification. In the neighbourhood of the nucleus of the cells we see roundish or spindle-shaped pigment corpuscles, brown in colour, which, on treating the preparation with alcohol, become green. Such chromatophores we also find, though certainly not in such large numbers, in the tissues of the stem, and in all cases we have to do with chromatophores containing a brown pigment which, under ordinary circumstances, completely masks their green colour.

In the brown Algæ belonging to the genus Fucus, the colour of

the chlorophyll is similarly disguised by the presence of a brown pigment, as I ascertained as follows. I collected in the neighbourhood of Cuxhaven a quantity of Fucus vesiculosus, packed it well so as to keep it fresh, and carried out the actual investigation on the next day. The younger parts of the plants were cut off and boiled for a short time in water. After pouring off the brown liquor the tissue of the algæ appeared green.* I now rinsed them with cold water, and treated them with alcohol. This quickly assumed a yellowish green colour; it was poured off and replaced by fresh alcohol. In this way we obtain a splendid green chlorophyll solution, which is highly fluorescent.[3]

We treat with alcohol for some time, leaves from the bud of Elodea, leaves of Funaria hygrometrica, or fern prothallia (the two last are specially to be recommended for our purpose). The objects become colourless, and, on microscopic examination, we perceive in the cells the protoplasmic matrix of the chlorophyll bodies freed from pigment. If we treat the preparations with a drop of a dilute aqueous solution of methyl violet, the decolourized chlorophyll corpuscles become deeply stained.

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[1] On the various relations here mentioned, see Strasburger's *Practical Botany* (Hillhouse).

[2] See Wiesner, Pringsheim's *Jahrbücher*, Bd. 8, p. 575.

[3] See Hansen, *Arbeiten d. bot. Instituts in Würzburg*, Bd. 2, p. 289.

6. Chlorophyll.

In recent years numerous attempts have been made, notably by Sachsse, Hansen and Tschirch, to isolate chlorophyll in a pure condition from green plant structures. These researches, as also the earlier ones of G. Kraus,[1] have shown that chlorophyll is a mixture of two pigments, viz., a blue-green one, cyanophyll, and a yellow one, xanthophyll. We shall not however here go into the details of the recent work, because the results still have more of a phyto-chemical than a physiological interest; and further, because the methods to be employed for isolating more or less pure chlorophyll preparations are of a highly complicated nature, and very tedious. We must, however, consider carefully the investigations of G. Kraus.

* The brown pigment mentioned is named phycophæin. The red pigment, soluble in water, which, together with chlorophyll, the Florideæ contain, is termed phycoerythrin. The blue-green fission algæ contain, in addition to chlorophyll, phycocyan.

So-called crude chlorophyll solutions we can ultimately obtain from any green plant structures; but in order to get a relatively pure extract, it is suitable to employ young wheat plants, or Elodea plants. We cut off the aerial parts of young wheat plants which have developed, say, six leaves, or we collect a moderate quantity of fresh Elodea plants, place the material (say 100–150 gr. of fresh substance) in a porcelain dish, and boil it for some time ($\frac{1}{4}$–$\frac{1}{2}$ hour) with distilled water on the water-bath. The liquor is poured off, the residue is washed several times with distilled water, and then, after being squeezed, is treated in a large flask with $\frac{1}{2}$ to 1 litre of 95 per cent. alcohol. The extraction proceeds pretty quickly, especially if we gently warm the flask. It is necessary to prepare the extract in the dark, since chlorophyll, as we shall see later, is very readily decomposed under the influence of light. The solution obtained has a splendid green colour.

Chlorophyll, as associated in the plant cells with a protoplasmic matrix, must on no account be regarded as a single chemical individual; it is a mixture of two pigments, yellow xanthophyll and blue-green cyanophyll, as we may readily prove by the following experiment.

Into a glass cylinder we pour a very concentrated alcoholic solution of chlorophyll, add water drop by drop, but not sufficient to produce any turbidity, treat with benzol, shake well, and let stand. The mixture rapidly separates into a lower alcoholic golden yellow solution of xanthophyll, and an upper blue-green solution of cyanophyll in benzol. The yellow pigment is more readily soluble in alcohol than in benzol, the blue-green one more readily soluble in benzol than in alcohol, and hence the separation of the two pigments.

In the cells of plant structures grown in the dark, and therefore yellow in colour, occur large quantities of etiolin grains (see 10). These consist of a protoplasmic matrix and a yellow pigment, which we can isolate by extracting with alcohol wheat or barley seedlings, *e.g.*, etiolated by growth in the dark. The extract has a beautiful yellow colour. We obtain the necessary plant material by sowing soaked wheat or barley grains on moist sawdust, and cultivating the young seedlings for about eight days in the dark.

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[1] G. Kraus, *Zur Kenntniss der Chlorophyllfarbstoffe.* Stuttgart, 1872.

7. The Absorption Spectrum and the Fluorescence of Chlorophyll.

To investigate the absorption spectrum of chlorophyll, we require a suitable spectroscope. According to circumstances we may use Bunsen's spectral apparatus, a direct vision spectroscope, a pocket spectroscope, or a micro-spectral apparatus, to be used in combination with a microscope. The micro-spectral apparatus, with scale tube and comparison prism, is to be obtained of excellent quality from Zeiss, of Jena, or from Seibert & Krafft, Wetzlar. The other instruments named are supplied, *e.g.*, by R. Muencke, Berlin, Luissenstrasse 58, and Geissler Nachf, in Bonn. An exact description of all the instruments would carry us too far. Detailed information may be found, *e.g.*, in Müller's *Lehrbuch der Physik und Meteorologie*, 1879, Bd. II., First pt., p. 206.

According to my own experience, it is most convenient to work with direct-vision spectroscopes. In investigations intended to determine exactly the position of the absorption bands, it is best to select instruments provided with scales, not graduated arbitrarily, but according to wave lengths. The exact focussing of the scale can then be effected in the simplest manner by means of the Frauenhofer's lines.

Considering first the micro-spectral apparatus represented in Fig. 6—as supplied by Seibert & Krafft, Wetzlar—it is to be noted that *t* represents the drum containing the slit and comparison prism. The screw *d* serves to narrow the slit, and the screw *h* to shorten it; *f* is the ocular tube, *o* the screw for focussing the slit to the eye of the observer; in the tube *rr* are the prisms, *s* is a mirror for conveying light to the comparison prism when it is inserted; *pp* is a perforated plate fixed at the side of the drum *t*, and provided with spring clamps. To obtain a comparison spectrum, the requisite substance is placed immediately in front of the hole in the plate *pp*. We shall not here describe the adjustment of the measuring apparatus, which may be attached laterally to the top of the spectroscope. The student is referred to Behren's *Hilfsbuch bei mikroscopischen Untersuchungen*, 1883, p. 121.

If we desire to investigate chlorophyll solutions, we prepare them in the manner described in 6. Here again we shall only experiment with the ordinary alcoholic crude chlorophyll solution, leaving out of account other chlorophyll preparations, which are

troublesome to make. The fresh solution, prepared in the dark, is poured into small glass bottles with parallel plane walls, and tightly-fitting stoppers, which may be obtained from Muencke, Berlin. These bottles are simply placed on the stage of the microscope, and we then study the spectrum. The chlorophyll spectrum exhibits seven absorption bands, as represented in Fig. 8. In particular, the band in the red is very characteristic, and can still be seen even with very dilute solutions. If we use fairly concentrated solutions, the bands I.–IV. still stand out distinctly, but the bands V., VI., and VII. are merged into a single end absorption. To see them, also, we must work with more dilute solutions, and employ direct sunlight. The results of the observations are to be noted on a scale on which the position of the Frauenhofer's lines is indicated. In exact investigations we

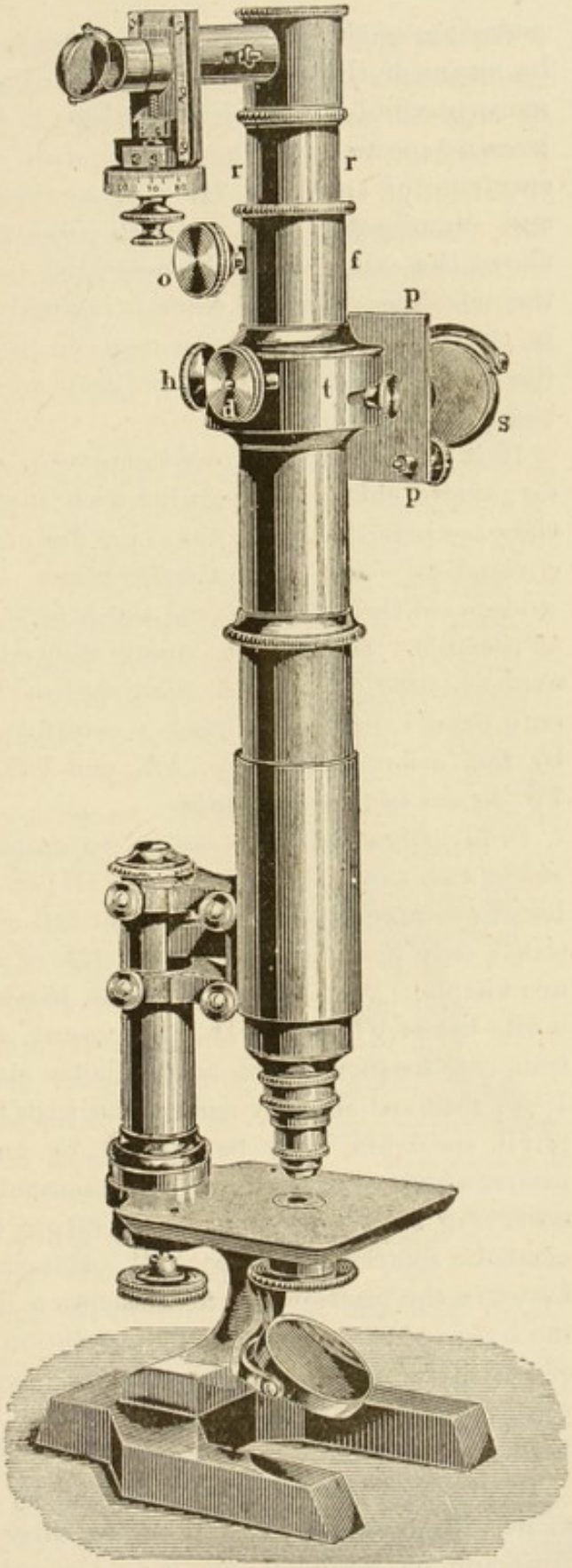

Fig. 6.—Microscope with complete microspectroscope.

make use of the measuring apparatus, which can be illuminated by means of the mirror attached to it. The comparison prism is more particularly of service when it is required to compare the known spectrum of one body with the not yet investigated spectrum of another. If, viz., we insert the comparison prism and illuminate it well, we shall perceive two spectra situated one above the other, and only separated by a fine black line. When the substances which we are investigating are identical, and also in the same state of concentration, the bands of absorption of the lower spectrum fall accurately on the prolongations of the bands of the upper.

If it is required to investigate thin sections of tissue, or even, *e.g.*, single chlorophyll grains with the micro-spectral apparatus, they are placed on the slide in a drop of water or glycerine, and covered as usual with a cover-glass. To focus the preparation, we remove the tube carrying the prisms, and open the slit as little as possible. In studying many chlorophyll-containing objects (I experimented, *e.g.*, with filaments of Cladophora), we perceive only band I. in the red, and a continuous end absorption formed by the fusion of bands V., VI., and VII.; the bands II., III., and IV. do not in general appear.

In investigating entire leaves we simply lay them on the object stage and use a low objective. If we illuminate our object by letting diffuse daylight or gaslight fall on the mirror of the microscope, only the bands I., II. and III. of the chlorophyll spectrum are visible. With direct sunlight, bands IV. and V. also appear, while bands VI. and VII., on account of the strong end absorption, can frequently not be distinctly made out. That the bands I.–V. observed actually correspond with bands I.–V. of the chlorophyll spectrum, may be proved by employing the comparison prism, which receives light from a special mirror, and with its help observing simultaneously the spectrum of a leaf, and that of an alcoholic solution of chlorophyll. Almost exact coincidence is then found in the position of the absorption bands of the two objects; the bands of the spectrum of the solution, as compared with those of the leaf spectrum, are merely displaced somewhat towards the violet, a phenomenon which is caused by the solvent (alcohol) used.

To investigate chlorophyll solutions by means of pocket spectroscopes, Bunsen's apparatus, or the direct vision spectroscopes of Hofmann or Steinheil, we pour the solutions into glasses with

parallel walls, such as are to be obtained, *e.g.*, from Muencke, Berlin (see Fig. 7); or what is best, transfer them to a hæmoscope (to be obtained of Desaga, in Heidelberg), so as to be able to examine in rapid succession layers of fluid of different thicknesses. If a gas or petroleum flame is used as the source of light, it frequently happens that we can only make out distinctly bands I. and II., or these together with band III.* Accurate spectroscopic investigation of chlorophyll can be made in direct sunlight. The spectroscope is placed in a dark room. The light enters through an opening in the window shutters, in front of which, in order to keep the direction of the rays of light constant, is placed a heliostat, whose mirror may be moved by hand, or better, rotated by means of clockwork. Good heliostats are supplied by Ehrhardt & Metzger, Darmstadt. In the dark room the walls, floor, ceiling, window shutters, tables, etc., are to be painted dull black. The absorption bands in the more strongly refrangible part of the chlorophyll spectrum are only to be seen clearly separated from one another when we experiment with comparatively dilute solutions. The results are to be noted down in accordance with the observations made with the help of the scale of the spectroscope.[1]

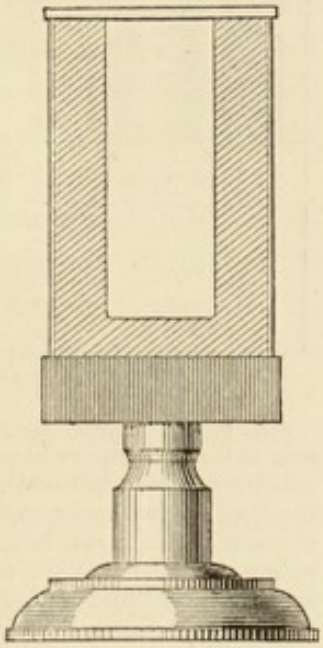

FIG. 7.—Glass vessel with parallel walls for the reception of fluids to be examined spectroscopically.

It has been indicated in 6 that normal green chlorophyll is a mixture of two colouring matters—xanthophyll and cyanophyll. The method to be employed for separating these two colouring matters from one another has also already been described. The solution of the yellow xanthophyll (at least the dilute solution) exhibits on spectroscopic examination only three absorption bands, all situated in the blue and violet.

* Very bright spectra are also given by the pocket spectroscopes of Browning, which are to be obtained, provided with scale, from Schmidt & Hänsch, in Berlin. As a source of light we may use an Argand burner with a tin chimney. These spectroscopes are now frequently employed for studying the absorption spectrum of chlorophyll solutions. It is possible, viz. with these, to determine quite accurately the position of the bands I.–IV. See Wegscheider, *Ber. d. Deutsch botan. Gesellschaft*, Bd. 2; and Vogel, *Praktische Spectralanalyse*, Nördlingen, 1877.

The blue-green cyanophyll has seven absorption bands—I. in the red, II. in the orange, III. in the yellow, IV. in the green, V., VI. and VII. in the blue and violet.

When we observe concentrated solutions of chlorophyll by reflected light, they are seen to have a red colour. The red colour

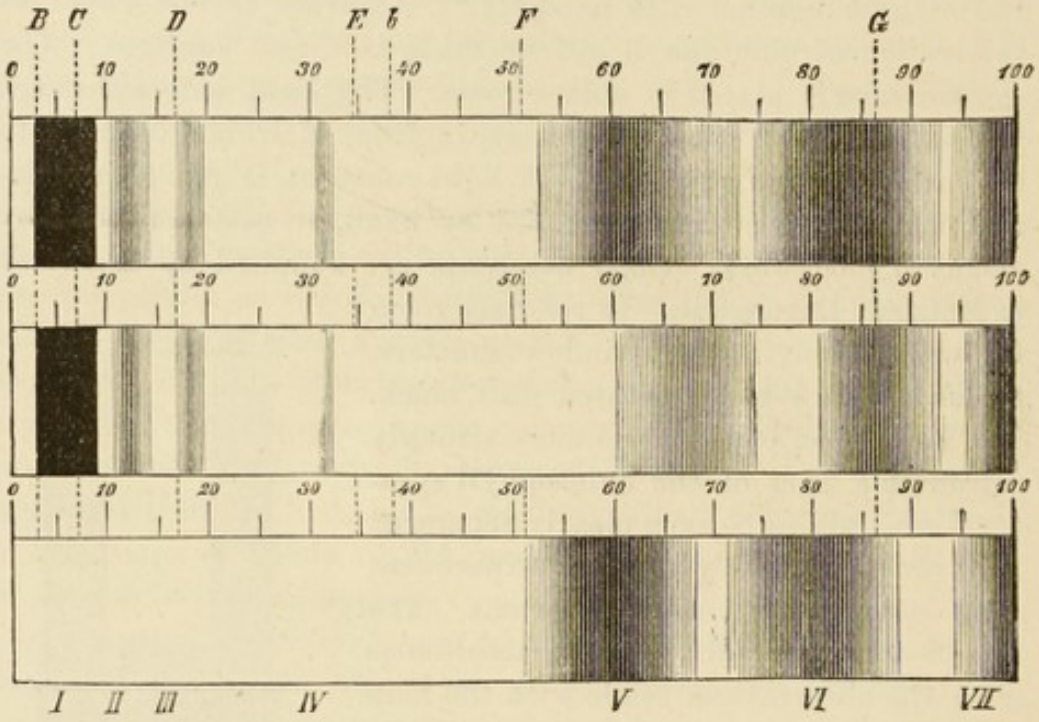

FIG. 8.—Absorption spectra of chlorophyll after Kraus. The uppermost spectrum is that of the alcoholic extract of green leaves, the middle one that of the blue-green constituent dissolved in benzol, the lowest one that of the yellow constituent. The absorption bands of the two upper spectra are given, in the less refrangible parts *B–E*, as from a more concentrated solution, in the more refrangible portions as from a dilute solution. The letters *A–G* indicate the position of the well-known Frauenhofer's lines of the sun's spectrum; the numbers *I.–VII.* denote, after Kraus, the absorption bands, proceeding from the red to the violet. The divisions 0–100 subdivide the spectrum into 100 equal parts.

of the solution of chlorophyll by reflected light is shown still more distinctly and beautifully when we throw a beam of light on the surface of the solution by means of a biconvex lens. Chlorophyll fluoresces with red light, as can thus easily be determined.

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[1] See Kraus, *Zur Kenntniss der Chlorophyllfarbstoffe*, 1872; Pringsheim, *Monatsber. d. Berliner Akademie*, 1874 and 1875, and *Sitzungsber. d. Berliner Akademie*, 1886; Hansen, *Arbeiten d. botan. Instituts in Würzburg*, Bd. 3, H. 1; Tschirch, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 1.

8. The Decomposition of Chlorophyll.

We place vigorous pot plants of Tropæolum majus in the dark, *e.g.*, in a cupboard. If the temperature is not too low, the leaves will already have experienced striking colour changes at the end of eight days. The older leaves, although still juicy, look yellow, while the younger are spotted, and the youngest are still quite green. The absence of light, as is shown by microscopical investigation of thin sections from the mesophyll of the Tropæolum leaves, has had an important influence on the chlorophyll grains. They have lost in size, and in place of the green pigment only a yellow one is now present.[1] If filaments of Spirogyra are for a long time (in my experiments for five or eight days, temperature 15–20°C) exposed in a glass containing some water to continuous darkness, the chlorophyll bodies undergo considerable changes. In many cells certainly green spiral bands are still present, in others, however, a breaking up of the chlorophyll bodies into irregular balls has already set in, the disorganisation being associated with a change in the colour of the pigment.

The chlorophyll in the cells also undergoes profound decompositions when brought in contact with acids. We lay filaments of Spirogyra or Zygnema (I used the latter with special success) in a mixture of 1 part of concentrated Hydrochloric acid, and 4 parts of water. The chlorophyll changes colour, and after some time (sometimes not for twenty hours) there appear in the chlorophyll bodies, especially at their edges, brownish or rust-coloured masses, decomposition products, due to the action of the Hydrochloric acid (Hypochlorin reaction).[2]

A crude chlorophyll solution (prepared by treating green plant structures with alcohol) similarly undergoes essential changes when treated with acids. If, *e.g.*, we treat such a solution with very dilute Nitric or Hydrochloric acid, its beautiful green colour is at once lost, and it assumes a brownish hue.

The crude alcoholic solution of chlorophyll of course contains, besides chlorophyll itself, a whole series of other substances, but we may nevertheless conveniently make use of it to prove that chlorophyll is highly sensitive to light. In the dark an alcoholic solution of chlorophyll remains unchanged for a long time; it only very gradually changes in colour. Diffuse light does not act very rapidly on a crude chlorophyll solution, but direct sunlight does; in direct sunlight such a solution very distinctly changes

colour in half an hour. It is also very instructive to investigate the influence of rays of different refrangibility on crude chlorophyll solutions. This is the first time we have been confronted with experiments on the influence of rays differing in refrangibility; and hence the method of procedure, of which we shall later have to avail ourselves frequently, must be described in detail.

An almost concentrated solution of Potassium bichromate, in not too thick layers, transmits red, orange, yellow, and a part of the green, almost undiminished in intensity. An ammoniacal

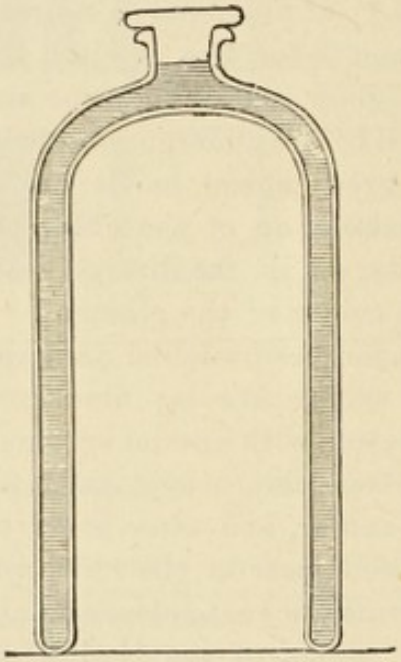

FIG. 9.—Double-walled bell glass to receive coloured fluids, represented in longitudinal section.

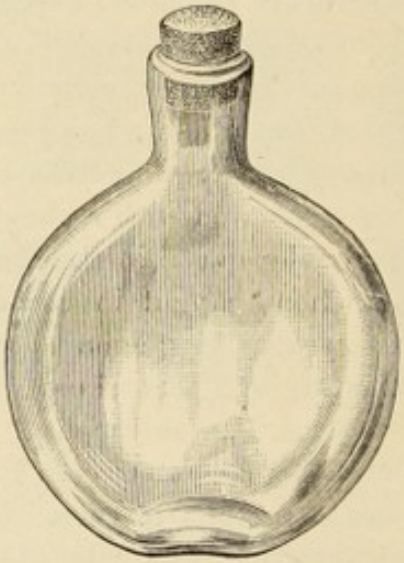

FIG. 10.—Glass bottle with parallel walls for the reception of coloured fluids.

solution of Copper oxide (prepared by dissolving Copper sulphate in water and adding excess of ammonia) absorbs the rays which the Potassium bichromate transmits, but does not arrest the remainder, viz., part of the green, the blue, indigo and violet. With these two solutions, therefore, we are in a position to decompose white light almost exactly into a more refrangible and a less refrangible half. To receive the coloured solutions we use very commonly double-walled bell-glasses (see Fig. 9).* The space between the two glass walls, and therefore also the thickness of the layer of coloured fluid, is generally about 1 cm. To prevent

* Such bell-glasses are to be obtained of Desaga, in Heidelberg.

the entrance of mixed white light it is convenient to place the bell-glasses on plates containing sand. The research material placed under them then receives only mixed yellow, or mixed blue, light. I also frequently used in experiments on the influence on physiological processes of rays differing in refrangibility, cardboard boxes pasted inside and outside with dull black paper, having for the back wall a well-fitting lid, while the front one is provided with a large hole. In front of this hole are fixed, in a suitable manner, glass bottles with parallel walls, which contain the coloured fluids (see Fig. 10). The solutions of Potassium bichromate and ammoniacal Copper oxide contained in the double-walled bell-glasses, or in the bottles just mentioned, are submitted to spectroscopic examination, the tube of the spectroscope being introduced into the bell in the one case, the bottle being brought close up to the slit of the spectroscope in the other. The solutions must be of such strength as in the one case to allow only the less refrangible rays, as above indicated, to pass; in the other, only the more refrangible rays.

If now crude chlorophyll solutions are exposed to the influence of mixed yellow and mixed blue light (direct sunlight), it is found that the less refrangible rays bring about the decomposition (change of colour) of the chlorophyll far more rapidly than the more refrangible rays. The so-called chemical rays, therefore, *i.e.* those which are able to decompose Silver chloride, participate only in a subordinate degree in the decomposition of chlorophyll, since the mixed blue light is rich in these chemical rays, while the mixed yellow light is poor in them, as we may readily ascertain by exposing photographic paper to the two kinds of light.[3]

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[1] See Sachs, *Botan. Zeitung*, 1864, p. 38.

[2] See Pringsheim's *Jahrbücher*, Bd. 12.

[3] For further literature, see Detmer, *Lehrbuch d. Pflanzenphysiologie*, 1883, p. 18.

9. The Autumnal Colouring of Leaves, and the Winter Colouring of Persistent Plant Structures.

Many leaves turn red in autumn before falling. This phenomenon may be particularly well studied in the leaves of species of Rhus, as also in those of Cornus sanguinea and Ampelopsis

hederacea. The leaves named exhibit the red coloration mainly on their upper surface, and microscopic investigation of delicate transverse sections teaches us in fact that it is particularly in the cells of the palisade parenchyma that the red pigment is contained. The pigment is dissolved in the cell-sap. The epidermal cells contain no pigment. In the autumnal yellowing of leaves the disorganising chlorophyll grains assume a yellowish colour, as can be determined for example by investigating maple leaves in autumn. As the change in colour of the leaves advances, the protoplasm and chlorophyll grains gradually dissolve; their substance passes over into the persistent structures of the plant, and finally there only remains in the cells xanthophyll in the form of small shining granules. Some leaves, *e.g.*, those of oaks, turn brown in autumn, a phenomenon which is to be referred to a browning of the cell-membranes as well as of the cell-contents.

The colour changes taking place in structures which last over the winter are also interesting. When the first frosts have set in, in autumn or winter, we observe that the surface of twigs of Thuja orientalis which is exposed to the light has assumed a brown colour.[1] This is due to a partial decomposition of the chlorophyll and the appearance of red pigments in the chlorophyll grains. If such brown Thuja twigs are brought into a warm room the red pigment disappears, and the twigs grow green again. Access of light is not necessary. Browned Thuja twigs which I kept in the dark at a temperature of 15–20° C. had already become green again at the end of eight days.

The winter reddening of persistent plant structures is to be referred to the formation of a red pigment soluble in the cell-sap, the chlorophyll grains remaining intact, and at most undergoing changes of position in the cells. If we examine in winter transverse sections of the leaves of Mahonia aquifolium, it is found that in particular the cells of the beautifully developed palisade parenchyma contain red pigment.[2]

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[1] A detailed account of the anatomical structure of twigs of Thuja (Th. occidentalis, however, not Th. orientalis) by Frank will be found in Pringsheim's *Jahrbücher*, Bd. 9, p. 159.

[2] On the winter colouring of persistent plant structures consult H. v. Mohl, *Vermischte Schriften*, p. 375, and G. Haberlandt, *Sitzungsberichte d. Akad. d. Wiss. zu Wien*, Bd. 72, Abth. 1, Aprilheft.

10. The Formation of Chlorophyll.

A few seeds of Lupinus are germinated in darkness, *e.g.*, in a cupboard, in garden soil * contained in flower-pots. It is by no means perfectly easy to make a place in which almost absolute darkness prevails, and if we wish to cultivate plants in absence of light, this must not be lost sight of. It is however sufficient for our purpose here to place the flower-pots in the cupboard, under an opaque cardboard receiver, and carefully plug the keyhole of the cupboard.

The hypocotyl and the cotyledons soon appear above ground, if the conditions of germination are moderately favourable. The cotyledons however are not green like those of Lupin seedlings grown in the light, they are yellow in colour. If we examine transverse sections of the cotyledons under the microscope, we see clearly the epidermis, the vascular bundles, and the leaf parenchyma. The cells of the last, especially the more peripheral ones, contain, besides other constituents, small yellow granules, the etiolin grains. If the seedlings raised in the dark are exposed to the light, they soon become green, and we now find chlorophyll grains in the cells of the leaf parenchyma. These are developed, under the influence of the light, from the etiolin grains, from which they differ not only in their green colour but also in their greater size. If seedlings of Phaseolus or Pisum are developed in darkness, they produce long white stems and small yellow leaves. I found that the leaves of pea seedlings, brought into the light after forming several internodes in darkness, did not all become green. The younger leaves certainly formed chlorophyll. The oldest ones, however, remained yellow.

Most plants form normal green chlorophyll only in access of light; some however become green even in the dark. If, *e.g.*, seeds of Pinus sylvestris are sown in garden soil, and germinated in absence of light (germination proceeds comparatively slowly), the root first breaks forth. Then the hypocotyl extends, at first however appearing with a knee bend, since the cotyledons still remain in the seed. Finally the cotyledons emerge from the

* It may here once for all be remarked that for culture experiments it is generally best to employ the dark, very humous soil used for greenhouse plants. The soil is just so much moistened that it can still be broken down between the hands into a finely crumbled mass, which is then thrown on to a sieve with meshes 1·5 mm. square, and riddled into the culture vessels.

ground, the hypocotyl then straightens, and germination is complete. The especially striking fact is that the cotyledons are green. I have frequently convinced myself that this greening of the Pinus cotyledons takes place in a dark space in which wheat seedlings do not grow green, but only develop a yellow plumule.

Seedlings of monocotyledons and dicotyledons (Phaseolus, Pisum, Raphanus, Triticum, Zea, etc.) grown in the dark, not only grow green when exposed to comparatively bright light; the formation of chlorophyll also takes place even in greatly weakened light, as we can readily ascertain by placing the objects at the back of a room, and here suitably screening them. We may further cause plants to become green by artificial light. Thus, for example, I placed wheat seedlings grown in the dark, and with plumules 2–3 cm. long, at a distance of 15 cm. from the flame of a petroleum lamp. The seedlings were in a crystallising glass with a little water. They became distinctly green, when thus illuminated by the light from the lamp, in a few hours; control plants, which were kept in the dark, did not become green.

It is further instructive to study the influence on chlorophyll formation of rays differing in refrangibility. For this purpose wheat seedlings, for example, raised in the dark, and with plumules about 2 cm. long, are placed in small glass dishes with a little water. These, or even small flower-pots, filled with soil, in which the seedlings have been raised, are now placed under double-walled bell-glasses, one of which is filled with a solution of Potassium bichromate, while the other contains an ammoniacal solution of Copper oxide (see 8). We can easily ascertain, by placing under the bells at the same time strips of photographic paper, that the mixed yellow light is almost completely free from the so-called chemical rays, while the light transmitted by the ammoniacal Copper oxide solution very rapidly brings about the decomposition of the Silver chloride. Having in the morning of a cloudy November day placed the apparatus at a distance of 5 m. from the window, in a room with a south aspect, at a temperature of about 20° C., I found at the end of twenty-four hours that the plumules of the seedlings under the influence of the mixed yellow light had become thoroughly green, while those which had been exposed to the action of the mixed blue light had a plumule light green in colour. If, on the other hand, we expose the apparatus to direct sunlight, the greening takes place more rapidly in the mixed blue light than

in the mixed yellow light. The result of the last comparative experiment however is open to doubt, unless very special precautions are taken, for I have satisfied myself that the air under a double-walled bell provided with ammoniacal Copper oxide solution becomes much warmer when exposed to direct sunlight, than the air under a bell-glass filled with Potassium bichromate. But still it certainly cannot be regarded as immaterial as to the result of the experiment whether we expose the seedlings grown in the dark to diffuse light or to direct sunlight. If wheat seedlings with yellow plumules are exposed to diffuse light, and others simultaneously to direct sunlight, the former rapidly become green, *e.g.*, in the course of three hours, while the latter produce normal chlorophyll far more slowly. Now we have seen in 8, that chlorophyll in alcoholic extracts from green plants very rapidly decomposes (changes colour) in direct sunlight, while diffuse light only very slowly induces changes, and this must be remembered in explaining the facts with which we have just become acquainted. In direct sunlight, whether it acts on the seedlings directly, or after traversing a solution of Potassium bichromate, seedlings become green slowly, since the chlorophyll as it is formed is in great part destroyed again. In diffuse light, as also in direct sunlight which has traversed an ammoniacal solution of Copper oxide, energetic decomposition of chlorophyll cannot take place, and so the chlorophyll formed rapidly accumulates in the cells of the seedlings. Feeble diffuse light hardly exerts any decomposing action on chlorophyll, and if seedlings are placed far away from the window under double-walled bell-glasses, they become green more rapidly in mixed yellow light than in mixed blue, since the former exerts almost exclusively its greater chlorophyll-forming power, its chlorophyll-destroying power standing very much in the background.[1]

To learn whether the dark heat rays can bring about the greening of seedlings grown in the dark, we place, *e.g.*, yellow-plumuled wheat seedlings in small glasses under double-walled bell-glasses filled with a solution of Iodine in Carbon bisulphide. If sufficiently strong, such a solution transmits no light rays, but permits the passage of the heat rays. The bell-glasses, surrounded at the bottom with sand, are exposed to direct sunlight or diffuse daylight; the research objects do not become green.

In order to study the relation between chlorophyll formation and conditions of temperature, it is convenient to employ, *e.g.*,

barley seedlings as research material. A good number of small flower-pots are filled with garden soil, barley grains are sown in each, and the pots are then put in the dark. When the plumules have reached a length of say 2 cm., we place one flower-pot before the window of a room with a north aspect, and in which the temperature is 6° C. Another pot is placed in a neighbouring room, in which the thermometer indicates 20° C. The plants are thus exposed to similar conditions of illumination, but to different temperatures, and it is found that the greening takes place far more slowly at 6° C. than at 20° C. At 30° C. the formation of chlorophyll proceeds somewhat more rapidly than at 20° C., at 37° C. slower again, and at 45° C. no greening takes place at all. To expose plants to temperatures of 30, 37, and 45° C., we put them into thermostats heated to the required temperatures. (A drawing and description of a suitable thermostat will be found in the second section, in connection with researches on root pressure.) It remains to be noted that the seedlings, before being illuminated, must be kept for some time in places of accurately known temperature, so that they, and also the flower-pots and the soil in them, may accommodate themselves to these temperatures. Judging from what was said above there are temperature-minima, -optima, and -maxima for the process of greening. The positions of these are by no means the same in different plants.[2]

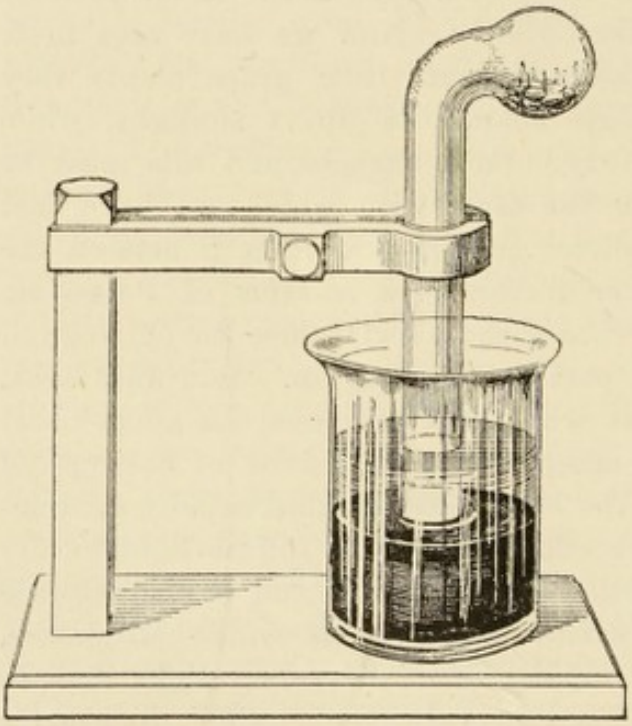

FIG. 11.—Apparatus for the culture of plants in a space devoid of Oxygen.

Lastly we will prove that the process of greening in the light cannot take place in absence of Oxygen. We fill two retort-like vessels (*a* and *b*) with distilled water which has been boiled and allowed to cool again in closed vessels, place in the water a

few wheat seedlings grown in the dark, and fix the vessels by means of a suitable stand with their mouths under mercury (see Fig. 11). We replace the water in *a* up to a certain point with Hydrogen, that in *b* by atmospheric air. If the vessels are now exposed to the influence of diffuse light, the seedlings in *b* rapidly become green, those in *a* do not.[3]

It may be remarked that the Hydrogen is prepared by the action of zinc on dilute Hydrochloric acid, and purified by passage through solutions of potash, Silver nitrate, and Potassium permanganate. The gas may be developed in a Kipp's apparatus (see Section III.).

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[1] See Wiesner, *Sitzungsberichte d. Akad. d. Wiss. in Wien*, Bd. 69, I. Abtheilung.

[2] See Wiesner, *Die Entstehung des Chlorophylls in den Pflanzen*, Wien, 1877.

[3] See Detmer, *Landwirtschaftl. Jahrbücher*, Bd. 11.

11. The Production of Oxygen in Assimilation.

Under the influence of light decomposition of Carbon dioxide takes place in the chlorophyll grains, Oxygen is set free, and with suitable research material can readily be detected as such.

We place in a glass say 200 c.c. of spring water, into which we lead not too much pure Carbon dioxide. The Carbon dioxide is prepared from marble by treatment with dilute Hydrochloric acid, and, before passage into the spring water, must be freed from any Hydrochloric acid carried over, by passing through a solution of Sodium bicarbonate. We first select for experiment a fairly long shoot end of Hippuris vulgaris, which, as shown in Fig. 12, is immersed below water with its base passing into a test-tube filled with water. The arrangement is now exposed for some time to direct sunlight. It is found that bubbles of gas spring from the cut end of the shoot, and gradually a considerable quantity of gas collects in the test-tube. If we close the mouth of the test-tube under water with the finger, invert it, and introduce a glowing splinter of wood, it at once bursts into bright flame. Our plant has therefore produced Oxygen.

This extremely interesting experiment for demonstrating the liberation of Oxygen by assimilating plants may also be made in a somewhat different way (see Fig. 13). A large number of shoots of Elodea or Ceratophyllum are placed under a funnel in a

glass filled with water holding Carbon dioxide in solution. Over the end of the tube of the funnel, which is below the surface of the water, is inverted a test-tube filled with water, and the whole is exposed to direct sunlight. The Oxygen, or, to speak more accurately, the air rich in Oxygen collects, as in the foregoing experiment, in the test-tube. Material for these experiments will be at our disposal for a considerable part of the winter if in autumn we put vigorous plants of Elodea into a large vessel of water, and place this at the window in a warm room, frequently renewing the water.

A glass cylinder is filled with spring water into which, if it is poor in free Carbon dioxide, we have led a small quantity of this gas. We now bring into the fluid a twig of Elodea or Hippuris, which may be bound to a glass rod (see Fig. 14), and shall observe that in the light bubbles of gas escape from the cut end of the twig. The number of bubbles, which consist of air rich in Oxygen, directly indicates the energy with which assimilation is proceeding in the green twig. In direct sunlight, for example, Elodea twigs frequently liberate such a rapid stream of fine bubbles that we cannot count them, or can only do so with difficulty. Other twigs of the same plant under the same conditions assimilate less energetically. The bubbles of gas escaping from the cut ends of Hippuris twigs are fairly large, and do not appear in such excessive numbers.

Without going into details regarding the relations between the intensity of the light on the one hand and the energy of assimilation on the other, it is easy to ascertain that the evolution of Oxygen by green plant structures proceeds more rapidly in bright diffuse light than in more feeble light. A shoot of Elodea bound on a glass rod is exposed under water to the influence of bright diffuse light. We count the number of bubbles liberated from the cut end in a particular time, *e.g.*, in one or in five minutes. We next place a ground glass plate in front of the apparatus, and it will be found that now fewer bubbles escape into the water in the unit time than before. If we shade the twig well, the assimilation, and hence also the production of Oxygen, falls off entirely.

Twigs of Elodea or Hippuris are exposed under spring water, into which if necessary some Carbon dioxide has been led, to very bright diffuse light. We count the number of bubbles escaping in a definite time. Over the vessel containing the research

material we now place a double-walled bell-glass filled with a solution of Potassium bichromate, and once again count the number of bubbles escaping. We then remove the bell-glass, and after again determining the number of bubbles escaping in bright diffuse daylight or in direct sunlight, replace it by a bell-glass containing ammoniacal solution of Copper oxide, and once again count the bubbles of gas. It is well to place a thermometer in

FIG. 12.—Apparatus for collecting the Oxygen produced by assimilating water plants.

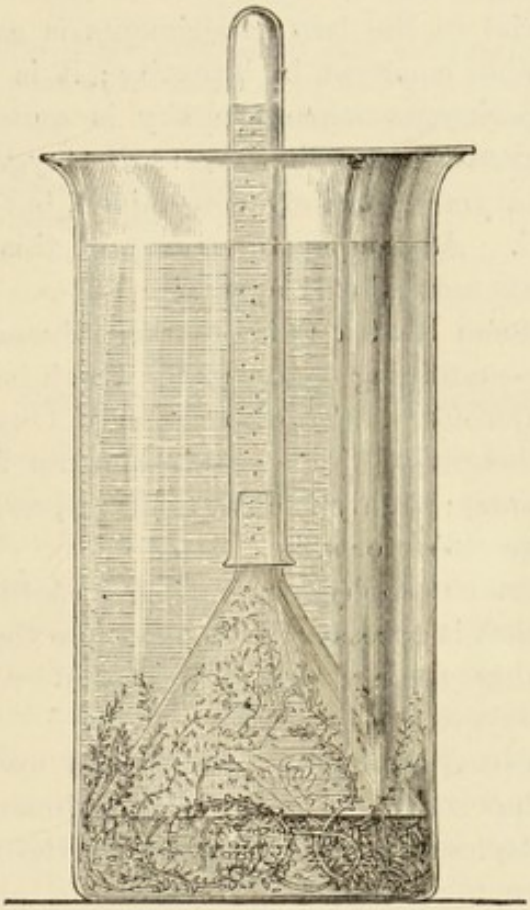

FIG. 13.—Apparatus for the same purpose.

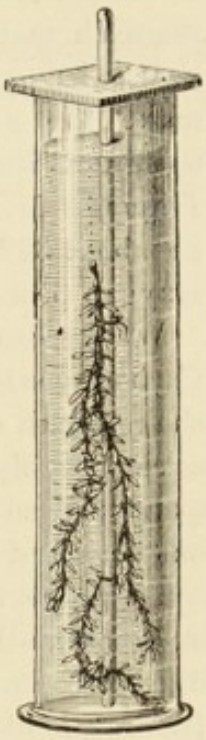

FIG. 14. — Apparatus for observing the liberation of bubbles of gas from assimilating water plants.

the water surrounding the research material so as to control the conditions of temperature during the separate stages of the experiment. Proceeding as above we easily succeed, as I have satisfied myself, in determining the interesting fact that the less refrangible rays transmitted by a solution of Potassium bichromate induce almost as energetic a liberation of Oxygen from green plant structures as does mixed white light, while the liberation of Oxygen proceeds under the influence of the more refrangible rays transmitted by an ammoniacal solution of Copper oxide only with very slight energy.[1]

Twigs of Elodea or Hippuris are placed, together with a ther-

mometer, in a beaker of water containing Carbon dioxide in solution. The temperature of the water may be, say, 12° C. We count the bubbles liberated in a certain time, *e.g.*, 1, 3, or 5 minutes, in access of light. We then warm the water in the beaker, without removing the plants from it, to a temperature of about 24° C. It will be found that the number of bubbles produced by the Elodea or Hippuris is now considerably greater than at the lower temperature. Care must of course be taken that the research material in the two experiments is exposed to light as nearly as possible constant in intensity; it is best therefore to make the experiments when the sky is quite cloudless. The optimum temperature for liberation of Oxygen in Elodea and Hippuris is not yet accurately determined, but may be taken to be about 32° C. At temperatures beyond this the liberation of Oxygen proceeds more slowly again.[2]

We expose some Elodea twigs to the influence of the light in Carbon dioxide-containing spring water which has previously been shaken with chloroform. The liberation of Oxygen continues for a remarkably long time (in any experiments for more than a quarter of an hour); finally it ceases entirely, owing to the poisonous action of the chloroform.[3]

To prove that chlorophyll-free plant structures are unable to assimilate, all that is necessary is to expose to the light, in Carbon dioxide-containing spring water, pieces of root for example. Liberation of Oxygen does not take place.

A very interesting method of proving the assimilatory activity of green cells has been introduced by Engelmann.[4] From a pure culture of Bacterium Termo, prepared in the manner to be described later, we take swarming individuals, place them on the slide in a drop of water, lay on the cover glass, and seal its edges with vaseline. With strong magnification we make out that the bacteria, being highly dependent upon the presence of Oxygen, soon come to rest; they only continue in movement in the neighbourhood of air-bubbles occurring in the preparation, and there also they ultimately come to rest. If however we introduce into the preparation with the swarmers an algal filament, the movement of the bacteria, as long as the alga is illuminated, continues without interruption. The Oxygen produced by the chlorophyll bodies acts as a stimulus, and occasions the movement, as also the direction of movement, of the bacteria. They collect in the neighbourhood of the Oxygen-distributing alga, and if, for example,

we experiment with threads of Spirogyra, the aggregation follows the course of the green bands. Our aërotropic swarmers thus form an excellent reagent for the presence of Oxygen. When the alga in the preparation is not illuminated, the movement of the bacteria ceases; with renewed illumination it at once recommences, since then Oxygen is once more set free in adequate quantity through assimilation.*

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[1] An accurate account of the influence of rays differing in refrangibility on the process of assimilation will be found in my *Lehrbuch der Pflanzenphysiologie*. On assimilation experiments in the objective spectrum, see Pfeffer, *Botan. Zeitung*, 1872, No. 23. As to the arrangements for the objective spectrum see the section on Heliotropic Nutations.

[2] See Heinrich, *Versuchsstationen*, Bd. 13, p. 136.

[3] See Detmer, *Landwirthschaftl. Jahrbücher*, Bd. 11.

[4] See Engelmann, *Botan. Zeitung*, 1881 and 1882.

12. Carbon Dioxide and Assimilation.

The whole of the Carbon dioxide which is worked up in assimilation is derived ultimately from the atmospheric air. This is a mixture of gases, which, apart from a few non-essential constituents, consists of about 79 per cent. by volume of Nitrogen, 21 per cent. by volume of Oxygen, and a small quantity of Carbon dioxide (in 10,000 volumes of air only 3 volumes of Carbon dioxide are present). That the air contains Oxygen may easily be demonstrated. We fasten a piece of cotton wool soaked in alcohol at the end of a thick wire bent twice at right angles. The alcohol is ignited and an inverted glass cylinder is placed over the flame. If we now quickly lower the mouth of the cylinder under water, the fluid at once rises in it, while the flame quickly goes out. The alcohol requires Oxygen for its combustion, and takes it from the air in the cylinder. As the Oxygen disappears, the water rises in the cylinder.

That atmospheric air contains Carbon dioxide is also easy to demonstrate. For this purpose a stream of air is led through clear baryta water by means of a water-air pump or a drop aspirator. The baryta water gradually becomes cloudy since the Carbon dioxide causes a precipitation of Barium carbonate. In

* Engelmann's method has also been employed to measure the intensity of Carbon dioxide decomposition and Oxygen production in different parts of the spectrum. For this purpose the mikrospectral objective of Zeiss is suitable.

accurate quantitative researches concerning the amount of Carbon dioxide contained in atmospheric air, the volume of air passed through the apparatus must be measured by means of a gas-meter. (For full instructions regarding the determination of the amount of Carbon dioxide in gas mixtures, see the third Section under Respiration.)

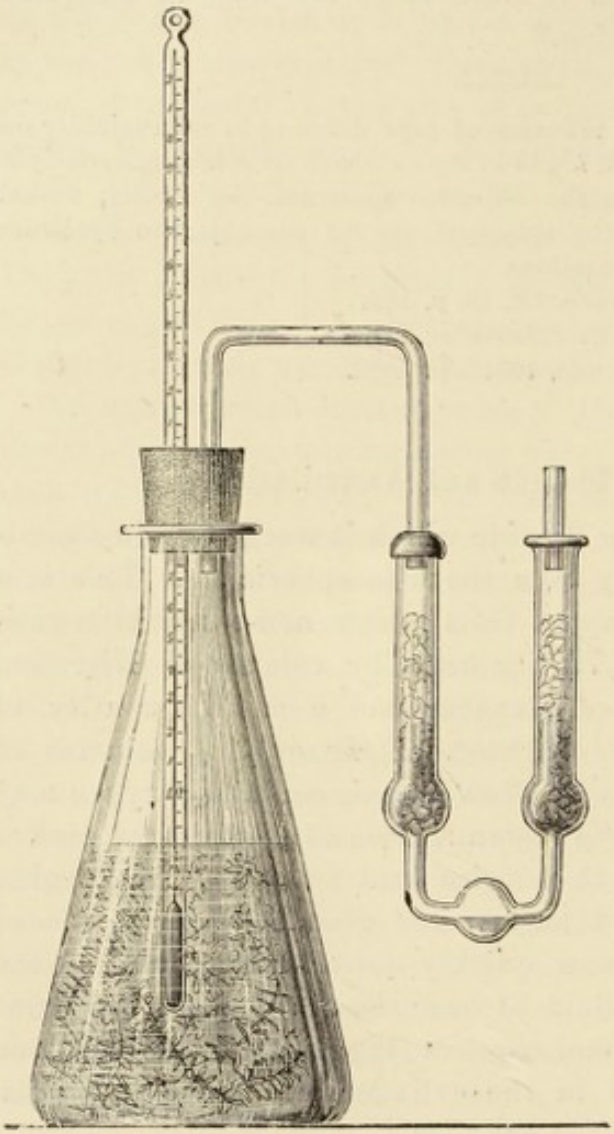

FIG. 15.—Apparatus for proving the fact that green plants can only produce Oxygen when Carbon Dioxide is at their disposal.

Water is able to absorb a not inconsiderable quantity of air. If a glass vessel about half filled with spring water is placed under the receiver of an air-pump, the air dissolved in the water at once escapes on working the pump, because the pressure of the air over the water is rapidly reduced. If to some spring water is added clear lime water or baryta water, a more or less considerable clouding of the fluid takes place, owing to the precipitation of Calcium carbonate or Barium carbonate as the case may be. This turbidity however does not conclusively prove that the water contains free Carbon dioxide in solution; it may be caused by Carbon dioxide present in loose combination in Calcium bicarbonate dissolved in the water.

Unless Carbon dioxide is present in the medium surrounding plants (air or water), no assimilation is possible, no formation of organic material, and naturally also no liberation of Oxygen. Proof of the fact that fresh organic material can be produced in

the green cells of plants only when Carbon dioxide is present shall be furnished later, but we will here perform an experiment which clearly shows that chlorophyll-containing cells only produce Oxygen when Carbon dioxide is at their disposal. Into a glass flask of about 400 c.c. capacity is poured 300 c.c. of spring water.* We now place in the water a considerable quantity of Elodea, and fit the flask with a perforated rubber stopper, through which passes one end of a glass tube, whose other end is connected with a U-tube containing fragments of pumice soaked in potash solution, together with fragments of caustic potash (see Fig. 15). The plants are not deprived of air because the water is in communication with the atmosphere through the U-tube. Access of Carbon dioxide from the atmosphere on the other hand is excluded. If we expose our apparatus to direct sunlight, we see that the plants keep up a rapid evolution of Oxygen at the expense of the Carbon dioxide dissolved in the water. We fix our attention on particular plants, and determine from time to time, say every half hour, the number of bubbles of gas which they liberate in the course of one minute. It is found that the evolution of Oxygen gradually becomes weaker and weaker, and finally (in my experiments after six hours) the production of Oxygen entirely ceases, since the supply of Carbon dioxide in the water is exhausted. If we now open the apparatus, and lead some Carbon dioxide into the water, the evolution of Oxygen by the plants under the influence of the light recommences.[1]

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[1] See Frank Schwarz, in *Unters. aus d. bot. Inst. zu Tübingen*, Bd. 1, p. 97.

13. Volumetric Relations of the Gas Exchange in Assimilation.

We conduct our experiments according to a method which has been accurately described by Pfeffer,[1] and which also Holle[2] employed. The apparatus is represented in Fig. 16. The most essential part of it is a glass tube about 360 mm. in length, expanded into a bulb towards its upper end. Of this tube the calibrated portion *c* occupies about 260 mm., and the bulb 70–75 mm., above which the tube ends in the narrow portion *a*

* A glass cylinder of small diameter is still more suitable.

which is open at the top. The capacity of the whole apparatus is about 115–120 c.c., of which about 75 c.c belong to the bulb. We take the free outer opening of the tube *a* as the zero for graduation, but the actual divisions only begin below the bulb, the diameter of the tube, as remains to be noticed, being here about 14 or 15 mm. The graduation may conveniently be carried to $\frac{2}{10}$ c.c., a detail which is not represented in the illustration.

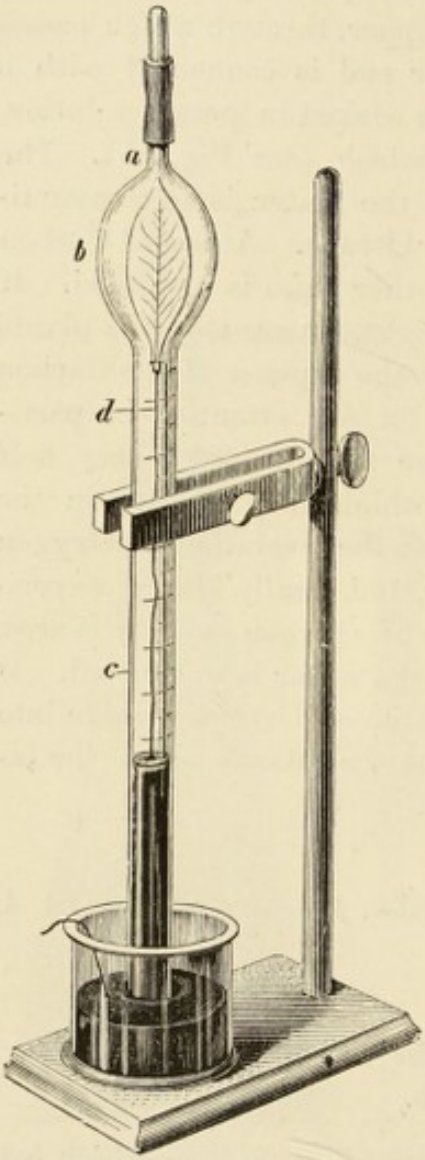

FIG. 16.—Apparatus for determining the quantity of Carbon dioxide which assimilating plants decompose.

In conducting volumetric observations on the gas exchange of assimilating leaves, it is necessary to cut away the leaf-stalk, leaving only a short piece to which is fastened a very thin iron wire *d*, the wire being run through the stump of the leaf-stalk, and wound several times round it. The leaf thus fixed on the wire is passed up into the bulb, an operation which is successfully effected by gently bending the edges of the leaf backwards, introducing it into the graduated portion of the tube, and pushing it up with a wooden rod. The apparatus is now fixed with its lower end dipping into mercury,* and we at once introduce into it over the mercury 0·3 c.c. of water, by means of a pipette bent round at its lower end and drawn out to a fine point. A piece of rubber tubing is slipped over the end of the tube *a*, which has so far been open. This is put into connection with a suction flask containing water. When we remove air from the flask by suction, the

* The mercury employed must be very pure. The purification of mercury which has been several times used may be effected as follows: We treat the metal in a thick-walled bottle with an equal volume of water, add to it a little Nitric acid, and shake well for a quarter of an hour or half an hour. We

mercury must rise in the apparatus. The connection tubing is closed at the proper time by means of a clip. We then introduce into it a piece of glass rod, ground and greased at its lower end, remove the clip, and push the glass rod home, so that its greased end fits closely to the top of the tube *a*.

The mercury having been sucked up into the tube as described, and the temperature of the gas having after some time become uniform, we can proceed to the necessary readings. We read off the volume of the gas, counting to the bottom of the water meniscus, and note also the height of the layer of water, and the height of the mercury above the surface. The height of the column of mercury expressed in millimetres, together with the mercurial pressure corresponding with the layer of water, gives the deduction to be made from the barometric reading. Temperature and barometric pressure must of course be accurately noted. From the gas volume observed is to be deducted the volume of the wire and leaf, which we determine in the usual way by immersion in water, and also 0·3 c.c. for the water meniscus. The volume is now reduced to 0° C., 1000 mm. pressure of mercury, and the condition of dryness :—[3]

$$V' = \frac{(V-m)\,(b-b'-b'')}{(1+0{\cdot}00366t^{\circ})}.$$

V' is the reduced gas volume; V denotes the volume observed; m, the meniscus correction; b, the barometric pressure; b', the pressure height to be deducted for the column of mercury in the tube; b'', the tension of aqueous vapour at a temperature of t°.

We now lead into the apparatus some purified Carbon dioxide (say 8 c.c.), once more determine the volume of gas in the apparatus, and again reduce as above. We can then at once find the volume of Carbon dioxide introduced by subtracting the first corrected reading from the second. In introducing the Carbon dioxide, and also in the previous operations, care must be taken to touch the apparatus as little as possible, so that the equalisation of temperature necessary before taking the readings may be effected as quickly as possible (in, say, ten to twenty minutes).

now carefully wash with water. If necessary, these operations are to be repeated. We then dry the mercury with blotting paper, heat it in a dish to 120° C. in a draught chamber, cover the dish with a sheet of paper, and allow to cool. Finally the metal is filtered through writing paper pierced here and there by means of a needle.

All analytical work must be performed away from direct sunlight. But when the Carbon dioxide has been led into the eudiometer we may expose the apparatus for some hours to direct sunlight, in order to ensure very vigorous assimilation. Immediately after exposure, the leaf is removed from the eudiometer, being rotated a little as it passes through the mercury, in order to liberate any bubbles of air which may be clinging to it. After about two hours, when the apparatus has quite cooled down, we read off the gas volume, and introduce some potash solution from a small pipette, by warming the pipette with the hand while keeping it closed at the top. After absorption of the Carbon dioxide which has escaped decomposition, the volume of the gas is once more determined.

For experiments concerning gas exchange in assimilation, leaves of Prunus laurocerasus or Nerium are suitable. Before introduction into the eudiometer, the leaves must have been exposed to the light so that they may contain no absorbed Carbon dioxide in their tissues. The leaves are left in the eudiometer exposed to the light for three to six hours, which is long enough, especially in direct sunlight, to ensure decomposition of a large quantity of the Carbon dioxide introduced. As regards the amount of Carbon dioxide to be led into the eudiometer, it may be remarked that 6–8 c.c. is sufficient. In many cases, when viz. the intensity of the sunlight to which the leaves are exposed is very considerable, it is desirable to hang over the eudiometer a double-walled bell-glass filled with water (see Fig. 9), to prevent undue heating of the leaf and of the gas in the apparatus. Or, if the light is too intense, we may shade the apparatus to some extent, for which purpose paper screens serve very well. If we desire to study the influence of coloured light on the rate of decomposition of Carbon dioxide, we suspend over the eudiometer double-walled bell-glasses filled with coloured fluids, and prevent access of light from below, say, by means of black oil-cloth. (See Pfeffer's cited treatise.)

We are at present, however, particularly interested only in the behaviour of leaves in mixed white light. Under the influence of such light notable quantities of Carbon dioxide are decomposed in a short time, and if we carefully carry out our investigations in the manner described, we shall be convinced that the volume of gas remaining in the eudiometer after exposure of the leaves to sunlight is as large as before exposure. Very small differences,

which come within the region of errors of observation, are of course to be disregarded. In assimilation, therefore, a quantity of Oxygen is produced which is exactly equal in volume to the quantity of Carbon dioxide decomposed.

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[1] See Pfeffer, *Arbeiten des bot. Instituts in Würzburg*, Bd. 1, H. 1.

[2] See Holle, *Flora*, 1877.

[3] See Bunsen, *Gasometrische Methoden*, 1857, and Hempel, *Gasanalytische Methoden*, 1890.

14. Macroscopical and Microscopical Detection of Starch in the Organs of Assimilation.

In very many green plant structures amylum is produced as the first easily visible product of assimilation. It is therefore one of the commonest tasks in plant physiology to detect this starch in the organs of assimilation, and this can be done either macrochemically or microchemically. We will first consider the former method.

The simplest method of investigation, first used by Sachs [1] in prolonged experiments, is as follows. We place the objects to be tested for starch (leaves of Tropæolum, Helianthus, Solanum, or Phaseolus are very suitable) for a few minutes in boiling water, and then transfer them to very strong alcohol at a temperature of 60° C. If we use a fairly large quantity of the alcohol, the chlorophyll of the leaves is generally very quickly and completely taken up, and in a few minutes the leaves become colourless. They are now placed in a solution of Iodine. This is prepared by dissolving a fairly large quantity of Iodine in strong alcohol, and then pouring the solution into distilled water till the fluid is about the colour of dark beer. We may also satisfactorily employ a solution of Iodine in Potassium iodide solution. The leaves are left in the Iodine solution for half an hour, an hour, or, if necessary, for several hours, till they undergo no further change of colour. We then remove them from the solution with the forceps, and lay them in a porcelain dish containing water. If starch is completely absent, the Iodine-saturated leaves are light yellow or buff-coloured. Small quantites of starch are indicated by darkish coloration of the leaves; large quantities by deep black coloration. If Iodine-saturated leaves, rich in starch, are left for several hours on a plate containing water, they often assume a

blue coloration. In experiments made by myself with leaves of Tropæolum, this came out very beautifully.

To determine microchemically whether starch is present or absent in the organs of assimilation, the material (*e.g.*, filaments of algæ or delicate transverse sections of leaves, etc.) is first laid in strong warm alcohol, in order to extract the chlorophyll. The bleached preparations are placed in a fairly strong solution of potash, either for a short time in a hot solution, or for twenty hours in a cold solution, carefully washed with water, treated with dilute Acetic acid so as to completely neutralise the potash, once more washed with water, and then mounted in a drop of iodised Potassium iodide solution (prepared by dissolving 0·05 gr. Iodine and 0·2 gr. of Potassium iodide in 15 gr. of water.)[2] The following method of detecting starch in green cells is also very convenient.[3] The research objects (with special success I used quite unprepared leaves of Elodea canadensis and Funaria hygrometrica) are either at once, or as is often necessary after extraction with alcohol, laid on the slide in a drop of chloral hydrate solution (5 parts of chloral hydrate to 2 parts of water) treated with iodised Potassium iodide solution, and at once observed. The chlorophyll is dissolved, the starch grains swell up somewhat, and they assume in contact with the Iodine solution a beautiful blue coloration, like starch-containing material placed in contact with the Iodine reagent after treatment with potash and dilute Acetic acid.

If we desire to satisfy ourselves that the starch formed by assimilation is not present in any part of a cell but in the chlorophyll bodies, we may select for examination Spirogyra, Zygnema, or leaves of Funaria hygrometrica, and avail ourselves of the last used method, with chloral hydrate.

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[1] See Sachs, *Arbeiten des botan. Instituts in Würzburg*, Bd. 3, p. 1.

[2] See Böhm, *Sitzungsberichte d. Akad. d. Wiss. zu Wien*, Bd. 22, p. 479, and Sachs, *Botan. Zeitung*, 1864, p. 291.

[3] See A. Meyer, *Das Chlorophyllkorn*, 1883, p. 28.

15. The Products of Assimilation.

There is no doubt whatever that starch always appears in the leaves of many plants as the first easily visible product of assimilation. On the other hand there are plants which under very favourable conditions for assimilation produce only comparatively

small or very insignificant quantities of starch, or even no starch at all. In the afternoon of a hot summer day we collect, *e.g.*, during the same hour leaves of Tropæolum majus, Phaseolus multiflorus, Helianthus annuus, of a Polygonum, of a Gentian, of Tamus communis, and of Allium Cepa. It is best if the plants from which we take the leaves have grown under conditions as nearly similar as possible, such as are afforded for example in botanic gardens. It is also to be observed that we must always work with fully developed leaves. On testing the material, in the manner described in 14, macroscopically or microscopically, we find that the leaves of Tropæolum, Phaseolus and Tamus contain copious quantities of starch in their green cells, while those of Helianthus already contain less. The quantity of starch in the leaves of Polygonum is still less, that in Gentian leaves very small, and the leaves of Allium prove to be completely free from starch.

To obtain further information, we perform the following instructive experiment. A large quantity of Helianthus tuberosus leaves are gathered on the afternoon of a hot summer day, and after removal of the leaf-stalks are cut into small pieces, and crushed between folds of linen in a hand press. We measure the volume of the dark-coloured juice obtained, boil it, allow to cool, then replace the water lost by evaporation, and filter. We prepare in exactly the same manner juice from leaves of Allium Cepa collected at the same time as the Helianthus leaves. We now determine the quantities of juice necessary for the reduction of 10 c.c. of Fehling's solution, and find that a large quantity of the Helianthus juice is required, but only a very small quantity of the Allium juice.* Leaves which produce large quantities of starch contain therefore but little glucose; leaves which form no starch are very rich in glucose. In fact, as Sachs[1] long ago declared, and as Arthur Meyer specially determined,[2] the glucose present in the leaves of Allium and other plants is to be regarded as a product of assimilation.

We may also conduct the investigations on the quantity of starch and sugar in assimilating leaves as follows, again employing

* Hand presses and cloths are to be obtained from G. Wenderoth in Cassel (see price list No. 2907). If the juices after filtering are very dark in colour, it is well to treat them with Lead acetate, filter, precipitate the lead with H_2S, and free the solution from H_2S by passing a current of air. On the preparation of Lead acetate, see C. Wolff, *Anleitung zur Untersuchung landwirthschl. wichtiger Stoffe*, 1875, p. 188. The Fehling's solution is prepared in the manner given in Section III.

on the one hand Helianthus leaves, and on the other Allium leaves. The material is dried as quickly as possible in a large drying chamber at about 80° C., then very finely ground and freed from water at 100° C. About 5 gr. of dry substance are treated in a beaker with 100 c.c. of water at 30° C., and after digesting for a few hours, we filter off the solution, and carefully wash the residue left on the filter-paper. The solution is made up to 200 c.c. and divided into two portions. In 50 c.c. of one we at once determine with Fehling's solution the quantity of reducing sugar present. The second portion is heated for a good time with a few drops of Hydrochloric acid, and then the quantity of sugar in this is determined. For further details see Section III. In this way we finally learn the quantity of reducing sugar in the leaves before and not till after inversion respectively. In order to determine also the quantity of starch in the leaves, the residue on the filter is rinsed in a flask with 200 c.c. of water, and further treated in the manner to be described in Section III. We finally determine with Fehling's solution the quantity of sugar obtained, and this serves as a measure of the quantity of starch in the leaves.[3]

This method may also be employed in investigating the specific energy of assimilation of leaves, or in studying the relationship between assimilation in the leaves and conditions of illumination, etc. We experiment, *e.g.*, with the leaves of Helianthus or Cucurbita. At five o'clock in the morning we remove one half from a few of the leaves without injuring the midribs. We lay the separated halves on a drawing board, cover them, with the exception of the stronger nerves, with thin templates of wood, about 50 or 100 sq. cm. in area, press these down, and with a scalpel cut out corresponding areas of leaf. The second halves of the leaves are not removed from the plant till after eight to twelve hours of assimilatory activity, and are then treated like the first, care being taken in each case to place the templates as nearly as possible symmetrically with respect to their position in the corresponding first removed halves. Immediately after cutting out the areas of leaf, we rapidly dry them at 80° C. In Helianthus annuus, 500 sq. c.c. of leaf surface weigh when dry about 4 gr. 5–10 gr. of dry substance then serve for the determination of the quantity of carbohydrate present (sugar, starch), and we shall find that the later cut halves are considerably richer in these than the first, if the conditions of assimilation during the

day have been favourable. While the leaves are assimilating, the whole of the material produced does not collect in the leaf. A portion of the carbohydrates migrates and is used up in respiration. This quantity must not be neglected. We may estimate it approximately by removing portions in the evening from leaves very similar to those used for the experiments already mentioned, and testing them for sugar and starch. The portions left behind are not cut till eight hours later, and these are also submitted to examination. Taking into account the loss by migration, we find, *e.g.*, that 1 sq. m. area of Helianthus leaf, under favourable conditions for assimilation, produces about 2 gr. of carbohydrate material per hour.[4] The quantity of carbohydrate which has migrated must naturally be added in the calculation to that produced in the daytime. It is noteworthy, as recent observations have taught, that even in Helianthus only a fraction (about $\frac{1}{6}$) of the carbohydrate produced in assimilation consists of starch.

The total quantity of the material formed by assimilation we can also discover approximately by taking, *e.g.*, from Helianthus plants, in the early morning and towards evening respectively 500 sq. cm. of leaf area, and thoroughly drying, first rapidly at about 80° C., and then, after powdering, at 100° C., proceeding in the same way with other portions removed in the evening and eight hours later respectively. The values for the gain in weight by day per 500 sq. cm., and for the loss in weight per 500 sq. cm. during the night are to be added. In this way we get a rough measure of the assimilatory activity of the leaves. These experiments, as also those previously described, are to be made on very bright sunny days and very warm nights.

We may to-day adopt the view that, even in very starchy leaves, starch is not directly produced in the green cells from the Carbon dioxide and water as the result of assimilation, but that first of all glucose is formed. (See my *Lehrbuch der Pflanzenphysiologie*, 1883, pp. 38 and 198.) In leaves rich in amylum this glucose, owing to specific peculiarities of their chlorophyll bodies, can easily be converted into amylum, while the formation of starch from the glucose developed in assimilation is more or less difficult in the leaf cells of other plants. In this connection it is naturally a fact of great importance that leaves have been caused to form starch at the expense of glucose conveyed to them from without.[5] I have not specially investigated the matter, but have satisfied myself that leaves can likewise form starch from cane-sugar

supplied to them from outside. Fresh pieces of Iris germanica leaf, about 10 cm. in length, were placed, without removal of the layer of wax, in a 20 per cent. solution of cane-sugar, contained in a shallow glass dish. The pieces of leaf floated on the fluid, and one of their surfaces did not come into contact with it at all. The vessel was covered with a sheet of glass, but between this and the rim of the vessel was placed a piece of cork so as to secure access of air. The material remained in contact with the cane-sugar solution for more than a week at a moderate temperature, and in darkness. At the beginning of the experiment, I tested some of the pieces of leaf macroscopically for starch; there was none present. Pieces of leaf which had remained for eight days in contact with the sugar solution gave a distinct starch reaction. It is to be observed that the pieces of Iris leaf, before being placed in the Iodine solution, must be treated for rather a long time with warm alcohol, to secure complete removal of their chlorophyll. The investigation may also be conveniently made with leaves of Nicotiana tabacum. Early in the morning a half leaf of the plant is tested macroscopically for absence of starch, the other half being laid in cane-sugar solution. After leaving this in the fluid for several days in the dark, it is found to contain starch in abundance.

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[1] See Sachs, *Handbuch d. Experimentalphysiologie d. Pflanzen*, 1865, p. 326.

[2] Arth. Meyer, *Botan. Zeitung*, 1885, Nr. 27.

[3] See also Saposchnikoff, *Berichte der Deutschen botan. Gesellschaft*, Bd. 9. Further see Brown and Morris, *Journal of the Chem. Soc.*, May, 1893.

[4] See Sachs, *Arbeiten d. botan. Instituts in Würzburg*, Bd. 3, and Saposchnikoff, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 8.

[5] See Böhm, *Botan. Zeitung*, 1863; A. Meyer, *Botan. Zeitung*, 1885, Nr. 27, and especially *Botan. Zeitung*, 1886, Nr. 5.

16. The Dependence of Starch Formation in Assimilation on External Conditions.

We sow some Phaseolus seeds in loose garden earth contained in flower-pots, grow the plants in darkness till the cotyledons have been robbed of a very considerable part of their stored reserve material, and now examine the primordial leaves macrochemically, after Sachs' method, or microchemically by treating thin transverse sections with chloral hydrate and iodised solution of Potassium iodide (see 14). Starch is not found in the mesophyll cells. If we now leave the plants for a few days exposed to

the light, they become green, their growth begins anew, and at the end of the time starch is to be detected in the cells of the leaves by macrochemical or microchemical tests.

If mature leaves of vigorous pot plants of Tropæolum or Phaseolus, grown in the light, are examined macrochemically or microchemically, starch will readily be detected in their cells. If the plants are now left in the dark, at a high summer temperature for a short time (say forty-eight hours), at a lower temperature for a longer time, the starch will disappear from the mesophyll of their leaves. If the plants are again exposed to the light for a few days, we can easily ascertain that their leaves are once more rich in starch. It is a good plan in comparative investigations concerning the influence of illumination on the origination and disappearance of starch in the cells of the leaf tissue, not to cut off entire leaves at the end of the separate periods, but pieces only. We are thus enabled to experiment with one and the same leaf throughout the entire period of investigation.

If we examine bud leaves of vigorous plants of Elodea canadensis, growing under normal conditions, we shall find starch present in the cells in large quantities. If the plants are placed in darkness, the starch completely disappears (in my experiments, at a high summer temperature, often within twenty-four hours, but certainly much more slowly at a lower temperature). Renewed illumination quickly causes reaccumulation of large quantities of starch in the leaf tissue. I have found it advisable not to treat the Elodea leaves with chloral hydrate and iodised Potassium iodide solution till they have been extracted with boiling water and hot alcohol for removal of their chlorophyll.

At a high summer temperature filaments of Spirogyra are free from starch after being kept in the dark for one to three days, as is shown by testing with chloral hydrate and iodised Potassium iodide solution. If we expose such starch-free filaments of Spirogyra to direct sunlight, they very soon, *e.g.*, even after half an hour, contain large quantities of starch, in consequence of the high temperature and the intense light. In diffuse light the formation of starch proceeds far more slowly.

To investigate the influence of light rays differing in refrangibility on the formation of starch in chlorophyll, it is convenient to employ as research material filaments of Spirogyra or Elodea plants which have been deprived of starch by being kept for a few days in darkness. The starch-free plants are placed under double-

walled bell-glasses in small dishes containing spring water, or are put into suitable boxes (see 8), and then exposed to light which has traversed solutions of Potassium bichromate and ammoniacal Copper oxide. We expose the apparatus to direct sunlight or to diffused light. From time to time, say every ten minutes if we experiment with direct sunlight, say every thirty minutes if with diffused daylight, we test filaments of Spirogyra or bud leaves of the Elodea for starch. It is found that just as evolution of Oxygen takes place far more actively in mixed yellow light than in mixed blue light, so also starch formation is brought about by rays of lower refrangibility far more copiously than by rays of higher refrangibility. In direct sunlight, however, the formation of starch in the chlorophyll goes on with tolerable rapidity, even under the influence of the mixed blue light. I found, *e.g.*, that filaments of Spirogyra, at first starch-free, contained considerable quantities of starch after exposure for thirty-five minutes, at a high temperature, to sunlight which had traversed the ammoniacal solution of Copper oxide.

It is instructive to investigate by Sachs' macrochemical method variegated leaves which have been vigorously assimilating. We may use for the purpose leaves of Acer negundo or Sanchezia (the last must be left for a fairly long time in the Iodine solution). It is seen that only the green parts of the leaf, not the parts free from chlorophyll, contain starch.

The following experiment which I made with pot plants of Tropæolum majus is particularly interesting since it teaches that starch formation in the mesophyll of the leaves is strictly localized, inasmuch as assimilatory activity is only exhibited by those parts of the leaf which are directly struck by the sun's rays, no starch being formed, *e.g.*, in artificially darkened parts of the same organ. Plants of Tropæolum are shaded for two days or more until macroscopic tests show that the mesophyll of the leaf has become free from starch. Then with pins we fasten to the upper and lower surfaces of full-grown leaves, exactly opposite one another, small discs of thick cardboard or felt, so that when the plants are again exposed to the light, only portions of the leaf surface will be illuminated. After some time (in my experiments after a day and a half) we test the partly shaded leaves macrochemically. The mesophyll of the artificially darkened regions of the leaf is starch-free; only the nerves contain starch. On the contrary the parts of the leaf which have been struck by the rays of light are

seen to be very rich in starch (see Fig. 17).

It is further important to satisfy ourselves that plants are unable to form starch when exposed to the light in an atmosphere free from Carbon dioxide, and indeed that the starch which may already be present disappears under these conditions from the chlorophyll bodies. We fill small flower-pots with ignited sand saturated with the ordinary food solution used for water-culture experiments, but diluted with water, and sow in them a few seeds of Raphanus sativus or other plants (with special success I experimented with Lepidium sativum). When the cotyledons have fully developed, the seedlings, in whose cotyledons we can easily detect large quantities of starch, are placed in the apparatus represented in Fig. 18. The glass

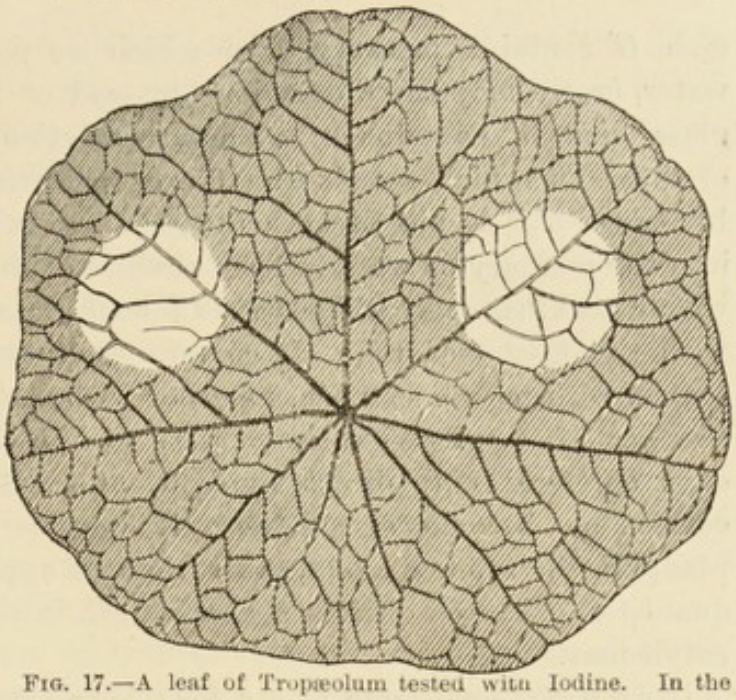

FIG. 17.—A leaf of Tropæolum tested with Iodine. In the white parts starch formation has been suppressed by cutting off the light, as described in the text.

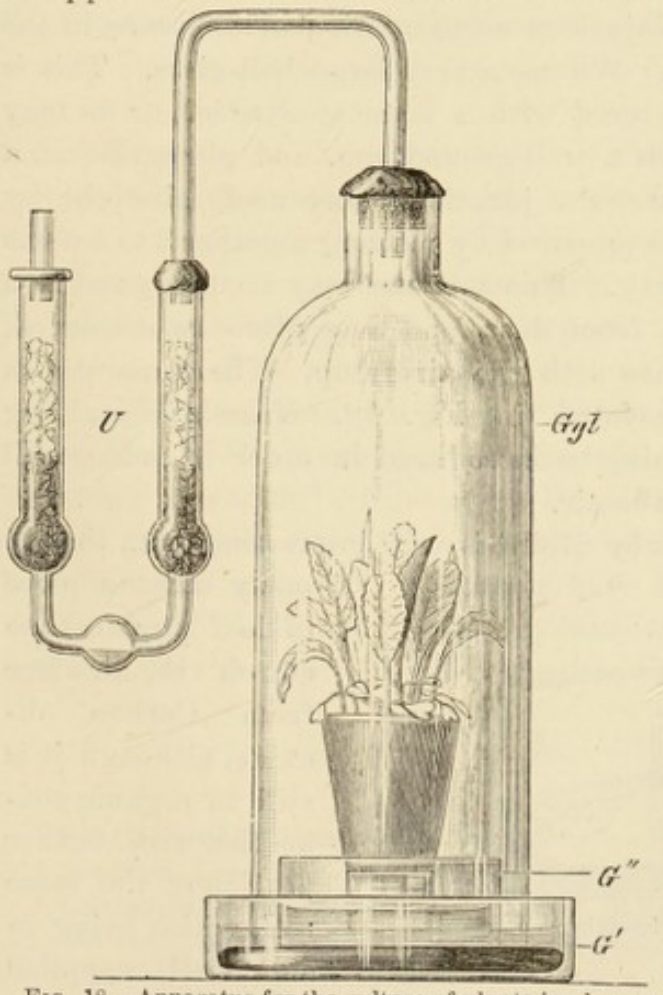

FIG. 18.—Apparatus for the culture of plants in absence of Carbon dioxide.

dish *G'* contains mercury, over which we pour a thin layer of water, or we may use water alone instead of the mercury. The glass dish *G''* contains strong potash solution, and in it is a slab of glass on which stands the flower pot with its plants. The bell-glass *Ggl* is placed over the seedlings with its rim dipping into the mercury or water. The tubulure of the bell-glass is fitted with a cork, through which passes a bent glass tube connected up with the U-shaped tube *U*, containing pieces of pumice stone soaked with potash solution, together with small pieces of caustic potash. If we expose the apparatus to the light for about two days (preferably to direct sunlight, at least periodically), the cotyledons become starch-free. If we now place the pot and plants in the window again, apart from the apparatus, considerable quantities of starch quickly collect afresh in the green cells of the cotyledons.[1]

If we wish to cultivate plants for a long time in an atmosphere free from Carbon dioxide, it is well to proceed as follows, so as to prevent too great and injurious accumulation of moisture in the air under the bell-glass. We use a very large bell-glass. This is dipped into mercury covered with a layer of olive oil, or we may employ a bell-glass with a well-ground rim, and place this on a rough ground glass plate, the junction being made air-tight by smearing with a mixture prepared by melting together 3 to 4 parts of lard, and 1 part of wax. This mixture may be preserved in a closed vessel, protected from dust, and may often be employed, even, *e.g.*, in experiments with the air-pump. The apparatus is put together as represented in Fig. 18. Vessels containing Calcium chloride may also be introduced in order to reduce still further the moisture of the air.

The fact determimed by Moll[2] is very interesting, viz., that in many cases a piece of leaf produces no starch in air free from Carbon dioxide, although it is still in organic connection with another portion of the same leaf, which being in air well supplied with Carbon dioxide is vigorously assimilating. We experiment

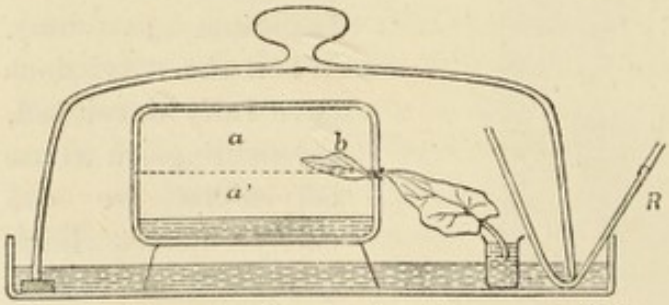

FIG. 19.—Apparatus for experimenting on assimilation. (After Moll.)

as follows: Two crystallising glasses are selected (see Fig. 19, *a* and *a'*), whose well-ground edges fit closely when put together. The edges of the glasses are now smeared with tallow, and starch-free leaves of Cucurbita Pepo or Vitis vinifera taken from shoots which have been kept in the dark for some time, so that they are starch-free, are placed between the edges in such a way that the tip of the leaf *b* is within the closed space, the base of the leaf and the leaf-stalk being outside. The lower glass contains potash solution. By gentle pressure the space between the glasses, say 600 c.c., is made air-tight. The leaf-stalk dips into a small vessel containing water. The whole apparatus is now placed under a large calibrated bell-glass of about 4,000 c.c. capacity. It rests on flat pieces of marble, and dips into water. By means of the bent tube *R*, we suck up into the bell-glass 200 c.c. of water, and then lead in pure Carbon dioxide to replace this quantity of water. The apparatus is now exposed for a few hours, possibly shaded, to direct sunlight. It is found, finally, that the portion of the leaf kept in the air charged with about 5 per cent. of Carbon dioxide, is very rich in starch, while no starch is produced in the terminal part of the leaf.

Experiments to demonstrate that conditions of temperature influence the formation of starch in the chlorophyll are best conducted in autumn or winter. Elodea plants freed from starch by being kept for some time in the dark, are placed in spring water in two rooms having the same aspect. One room is kept at a temperature of about 6° C., the other at a temperature of about 20° C. The temperature of the water in the one case is kept constant at 6° C. (if necessary by introducing fragments of ice), in the other case at 20° (if necessary by adding warm water). From time to time (say every 30 minutes) we test the leaves for starch, and find that although the plants have been exposed to similar conditions of illumination, larger quantities of starch have formed more rapidly at the higher temperature than at the lower.[3]

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[1] See Godlewski, *Flora*, 1873, p. 382.

[2] See Moll, *Landwirthschaftl. Jahrb.*, Bd. 6, p. 345.

[3] Literature respecting starch formation: Sachs, *Botan. Zeitung*, 1862, Nr. 44; the same, 1864, Nr. 38; *Arbeiten d. botanischen Instituts in Würzburg*, Bd. 3, H. 1; G. Kraus, *Pringsheim's Jahrbücher*, Bd. 7, p. 511; Nagamatsz, *Beiträge zur Kenntniss d. Chlorophyllfunction, Dissertation, Würzburg*, 1886.

17. The Stomata and Assimilation.

Recent researches of Stahl clearly indicate that the Carbon dioxide required in assimilation enters the leaf under normal circumstances through the stomata (the case is different if the quantity of Carbon dioxide in the air surrounding the plants is increased), and that at most only traces of the gas find their way into the interior of the tissues through the cuticularised membranes. I repeated Stahl's experiments with cut twigs of Lonicera tatarica, and pot plants of Phaseolus multiflorus. The material was first kept for twenty-four hours in the dark; the leaves had by this time become free from starch. Now a few of the leaves, without being cut off, were painted on one half of the lower side with a warmed mixture of 1 part wax and 3 parts cacao butter, in order to close the stomata, and then at once exposed to the sunlight. It is advisable to leave on the twigs or rooted plants only a few leaves so that they transpire feebly and do not wither. After 4 to 6 hours the leaves are dipped into cold spring water, the crust of fat is dissolved from their under sides, and after extraction with alcohol they are submitted to the Iodine test. The portions of the leaves whose stomata were sealed, have formed no starch, while the assimilation in the other halves of the leaves has taken place normally (see Fig. 20).

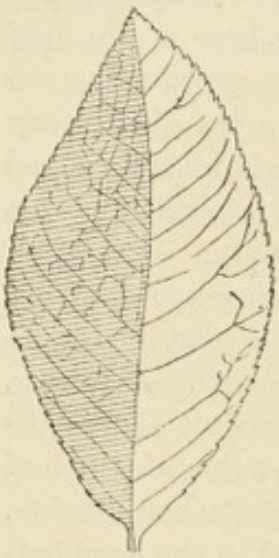

Fig. 20.—Young leaf of Prunus padus. The right half has been smeared below with cacao butter. The drawing has been made from material subjected to the Iodine test. (After Stahl.)

If the upper surface of the leaves is coated with the cacao wax, the assimilation is not much reduced, since large quantities of Carbon dioxide pass into the interior of the leaf through the stomata of the lower surface. This experiment at the same time teaches that the presence of the coating of wax does not in itself in any way injure the leaf.

We cut twigs of Lonicera tatarica, Syringa vulgaris, or Sambucus nigra, and place them for twenty-four hours in the dark to deprive them of starch. Small fragments are removed from some of the leaves, and it is determined that starch is absent. When this is the case we cut a few leaves from the twigs, and divide them into halves,

by a cut running alongside the midrib. The halves provided with midrib are at once placed in a moist atmosphere under a bell-glass, in order to keep them turgescent; the cut surface of the leaf-stalk dips into water. The other halves are placed in a poorly lighted part of the room till they have become somewhat limp. Both sets are now placed in a ventilated glass receptacle, the former with their stalks still in water, and exposed to direct sunlight. In order to prevent undue warming, we interpose in the path of the sun's rays a parallel-walled glass vessel containing water. After they have been isolated for about three hours, the Iodine test is applied to the objects. The limp halves have formed no starch, the others kept fresh have formed large quantities of it. The withering in this case—and the like is to be observed in very many plants—has caused the stomata to close, and consequently the admission of Carbon dioxide and activity of assimilation has practically been brought to an end. If we experiment with halved leaves of Hydrangea hortensis we find that even the limp halves, if not too far gone, also produce starch in the sunlight. In this plant namely the stomata do not close when the tissue withers.

We grow seedlings of Zea Mais, some in normal food solution, others in a food solution to which we have added 0·5 per cent. of common salt. The former thrive well, while the latter, although their tissues do not undergo any striking changes, are completely retarded in their development. In the leaves of the former large quantities of starch and glucose can easily be detected; the latter are completely wanting in these substances. (The examinations for starch and sugar are to be made when the plants are a few weeks old.)

These facts, first established by Schimper, find their explanation, as Stahl found, in the fact that the Maize plants which take up Sodium chloride do not assimilate, since their stomata, owing to the influence of the salt, close. The absorption of Carbon dioxide by the plants is then inadequate. By means of the Cobalt test which will be fully discussed in the section on Transpiration, the closure of the stomata can in fact be easily determined.[1]

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[1] See Stahl, *Botan. Zeitung*, 1894; Nagamatsz, in *Arbeiten d. botan. Instituts in Würzburg*, Bd. 3; Schimper, *Sitzungsber. d. Akadem. d. Wiss. zu Berlin*, 1890, Sitzung V., 31 July.

II.—THE PRODUCTION OF PROTEIDS IN PLANTS.

18. The Nitrogenous Food of Lower Organisms.

It is very important to prove that the cells of many plants have the power of producing nitrogenous organic bodies, *e.g.* proteids, from non-nitrogenous organic substances (*e.g.* sugar) and nitrogenous inorganic material. We experiment with yeast (Saccharomyces cerevisiæ).[1]

We take three flasks, *a*, *b*, *c*, of about 200 c.c. capacity. In *a* we place 100 c.c. of distilled water, in *b* 100 c.c. of Pasteur's food solution (1,000 parts by weight of this solution consist of 838 parts of water, 150 parts of grape-sugar, or sugar candy [in my experiments I mostly used the latter, since it is more readily obtained pure than grape-sugar], 10 parts of Ammonium tartrate, 0·2 parts of Magnesium sulphate, 0·2 parts of Calcium phosphate, and 2 parts of acid Potassium phosphate) *; in *c* 100 c.c. of a fluid having the same composition as Pasteur's solution, except that it contains no Ammonium tartrate. After plugging the mouths of the flasks with cotton wool, we boil the three fluids for some time, in order to sterilise them as completely as possible, and after allowing to cool remove the plugs for an instant, and introduce a small quantity of yeast into the fluids. We use 1 or 2 c.c. of yeast-containing fluid (see Appendix). The vessels, closed with cotton wool, are exposed to a temperature of about 25° C. and put aside either in darkness or in the light, but frequently agitated. The fluid in *b* rapidly becomes very turbid, and very many new yeast cells are produced, which settle in a mass at the bottom. The fluids in *a* and *c* either do not become turbid at all, or very slightly. Since it is very difficult to free yeast of all foreign substances, multiplication may possibly go on to a very slight extent in *a* and *c*, but at all events the copious multiplication which takes place in *b* (as may be more exactly determined by filtering and then drying and weighing the yeast obtained) shows that here alone are the normal life conditions of the fungus fulfilled. The formation of new cells would necessitate the elaboration of nitrogenous organic substances,

* According to the researches of Molisch, fungi also require for normal development to be supplied with iron. Generally sugar contains a certain quantity of iron. To make quite sure, however, we may add to the Pasteur's solution 0·01 per cent. of Ferric sulphate.

since the protoplasm of the cells is of course rich in such bodies, and only non-nitrogenous organic substances and ammonia have been employed.

While the higher plants can readily utilise Nitric acid for the formation of proteids, the yeast cell is not in a position to make use of it. If we replace the Ammonium tartrate of Pasteur's food solution by Potassium nitrate, and add yeast cells to the fluid, the fungus behaves exactly as it does in a solution to which no nitrogenous substances at all have been added. On the other hand, by experiments in which we replace the Ammonium tartrate of the Pasteur's solution by peptone, we can ascertain that this last body is a more favourable source of Nitrogen for the yeast plant than ammonia.

That not only yeast cells but also other organisms not possessing chlorophyll can prepare proteids from sugar and ammonia can readily be determined. Two small vessels, *a* and *b*, are procured. Into *a* we pour 25 c.c. of Pasteur's solution, into *b* 25 c.c. of a fluid of similar composition except that the Ammonium tartrate is absent. We now place both vessels under a bell-glass, and let them stand for about eight days. The fluid in *a* very soon becomes turbid, owing to copious development of bacteria. Other organisms also may appear (in my experiments, for example, the red Saccharomyces glutinis). The fluid in *b* becomes only slightly turbid since there is no source of Nitrogen. Feeble indications of the life of the germs which are present may appear, since the fluid may perhaps absorb some ammonia from the air.

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[1] See A. Mayer, *Untersuchungen über die alkoholische Gährung*, 1868, and *Lehrbuch der Gährungschemie*, 1874, p. 108.

19. Can Seedlings Make Use of the Free Nitrogen of the Atmosphere for the Formation of Proteids?

Plant cells can produce proteids from non-nitrogenous organic material and nitrogenous inorganic compounds (Nitric acid and ammonia). It is another question whether free atmospheric Nitrogen can be employed for the same purpose, and this question is not only of theoretical but also of great practical interest. We first of all make experiments which teach that seedlings under particular conditions neither take up free Nitrogen nor experience loss of Nitrogen in their development.

Pea and wheat plants are very suitable as research material. We first provide ourselves with well developed and thoroughly good seeds, and determine their dry weight and also the quantity of Nitrogen they contain.[1] A few wheat grains (say 30) or pea seeds (say 6) are accurately weighed. From their weight we can easily determine the quantity of Nitrogen they contain on the basis of the Nitrogen estimations above mentioned. The objects are placed in a small glass dish with a little freshly prepared distilled water. The dish is left for about twenty-four hours under the bell-glass of the apparatus to be described immediately, and at the end of this period the small quantity of water still left is poured off from the seeds and evaporated to dryness in a porcelain dish on the water-bath. The dish with the residue is placed in a dessicator. The soaked seeds are now brought to further development in the apparatus represented in Fig. 21. On a ground glass plate stands a beaker *B*, over the top of which is bound a piece of netting or a piece of perforated parchment paper. The beaker contains a food solution made with freshly prepared distilled water, and in which are present all the food stuffs except Nitric acid and ammonia (see 22). The seeds are placed on the netting or parchment paper, in place of which we may use a perforated silver plate, so that the developing roots grow down into the food solution. The ground edge of the very large bell-glass *G* is cemented perfectly air-tight to the ground glass plate. This is best effected by means of a cement prepared by melting together 1 part of wax and 3 to 4 parts of lard. The bell-glass may also be closed below by placing it in a large dish of mercury. The mercury must, however, be covered with water so as to prevent injury to the seedlings from the vapour of the

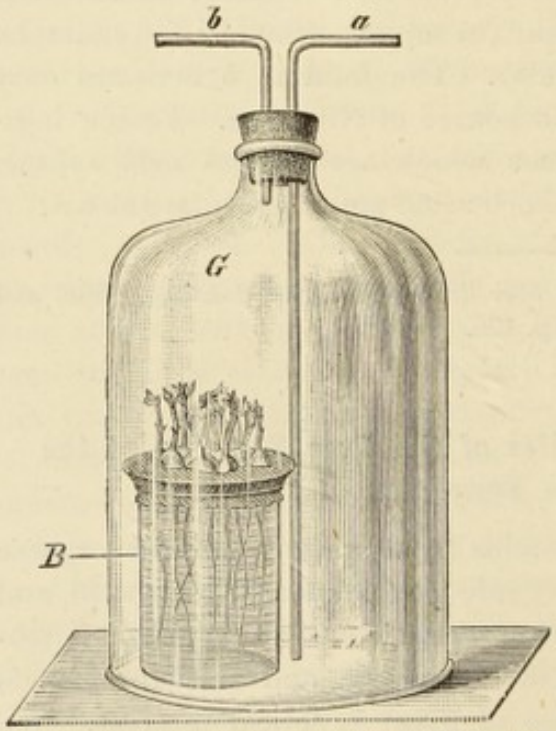

FIG. 21.—Apparatus for the culture of plants in the absence of all nitrogenous compounds.

mercury. It is often advisable also not to place the swollen seeds at once on the covering of the beaker, but first let them germinate under the bell-glass on moist wool. The tubulure of the bell-glass is provided with a 2-holed rubber stopper, through which pass the two tubes *a* and *b*, both bent at right angles. Through *a* is led atmospheric air, which however has first been freed from all nitrogenous compounds, and again saturated to some extent with aqueous vapour. For this purpose the air before it passes into *a*, is led through a series of wash bottles, of which the first contains a solution of Sodium bicarbonate, the second pumice stone soaked with Sulphuric acid, and the third water. The tube *b* is connected up with an aspirator, but it is well to interpose between this and *b* another small bottle containing Sulphuric acid, so that the air under the bell-glass is not in direct communication with the atmosphere at all. (Respecting the method of using the aspirator, see the section on Respiration.)

If a continuous slow stream of air is led through the apparatus from the time the seeds are set to soak, the plants being exposed to bright diffuse light, they develop as well as is possible in absence of Nitric acid and ammonia. The experiments are carried on for, say, fourteen days or even longer, air being passed through the apparatus day and night during the whole time. It is then necessary to determine the quantity of Nitrogen contained in the seedlings produced. The plants are reduced to a pulp in a porcelain dish. The weight of the dish, and also that of a glass rod used for stirring the pulp must be determined. We then place the dish on a water bath and add to it the balance of the food solution, evaporated to a small bulk, in which the roots of the plants developed, together with the residue from the soaking water and from the water used for washing out the glass wool on which the seeds may have been germinated. When the mass in the dish has become fairly dry, it is placed for some time in a drying chamber at a temperature of about 50° C. The dish, loosely covered, is then exposed to the air for twenty-four hours. We ascertain the weight of its air-dry contents, and then at once determine the corresponding dry weight. Nitrogen determinations are also to be made.* If we now finally compare the

* In estimating the Nitrogen in the whole of the research material, it is specially desirable to dry the macerated seedlings, together with the residues from the soaking water and food solution, in Hoffmeister dishes (to be obtained of Muencke, in Berlin).

amount of Nitrogen in the seeds used with that in the plants obtained, we shall find, provided the experiments have been carefully carried out, that the differences are at most such as are due to small errors of experiment. The plants are not in a position, under the conditions specified, to utilise the free Nitrogen of the atmosphere for the formation of proteids.[2]

Similar experiments are to be made if we desire to satisfy ourselves that seedlings vegetating in darkness neither take up free Nitrogen from the air nor suffer loss of Nitrogen. We have only to take care that the seedlings are not exposed to rays of light.[3]

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[1] On Nitrogen determinations according to the methods of Dumas and Kjeldahl, with the mode of preparing the necessary normal acids, etc., see König, *Anleitung zur Untersuchung landwirthschaftl. wichtiger Stoffe*, 1891, pp. 150 and 682, and Fresenius, *Quantitative Analysis.*

[2] This fact has been particularly determined by Boussingault (see *Compt. rend.*, T. 39, p. 601).

[3] I have collected together my own results and those of other observers on this subject in my *Vergleichende Physiologie des Keimungsprozesses der Samen*, 1880.

20. Bacterium Radicicola and the Papilionaceæ.

The question of the significance of free Nitrogen as food for plants has frequently been experimentally investigated. Till recently, bearing in mind the results of Boussingault's researches and those of other investigators, scientists in general held the view that plants were unable to employ elementary Nitrogen in the process of nutrition. The conditions under which Boussingault's experiments were made actually excluded the possibility, as we now know, of free Nitrogen being worked up in the cells. Under other conditions, however, the process can take place, and the great honour of having determined this with certainty belongs especially to Hellriegel.[1]

To obtain general information we make the following experiment, which, however, can only be conducted during the warmer part of the year.

A number of cylinders 24 cm. in height and 14 cm. in diameter, capable of holding about 4–5 kg. of sand,* are covered at the bottom to a depth of 3 cm. with washed and ignited fragments of

* In my experience good results are also obtained with cylinders holding only 2½ kg. of sand.

quartz. On this we put a thin layer of unsized cotton wool, and fill the cylinders with a quartz sand, the particles of which are mostly 0·2–0·4 mm. in diameter.* Before being filled into the cylinder, the sand is prepared as follows:—Every kilogramme of dry sand to be used is mixed with 4 gr. of Calcium carbonate, 0·150 gr. of Potassium phosphate, 0·070 gr. of Potassium chloride, 0·070 gr. of Magnesium sulphate (the last three salts dissolved in 150 c.c. of water) and a little phosphate of iron. The sand is now in the moist state crumbled into the cylinders and gently pressed down from time to time. The same quantity of sand is introduced into each cylinder. For investigation we select oats and peas. The seeds must be very normal and of medium weight. They are germinated between folds of blotting-paper, and the seedlings are put into the sand, twelve oat seedlings or six pea seedlings to each cylinder. After a time we remove six out of every set of twelve oat seedlings, and three out of every six pea seedlings, taking great care that remnants of seed and roots are not left behind in the sand. The cylinders are now left with six of the best developed oat seedlings or three pea seedlings. The latter are at once provided with supports. The cylinders are set in a sunny position in the garden, being brought under cover only in rain or strong wind, or on very hot days. Every day the cylinders are weighed, and the water lost by evaporation is replaced. Besides the cylinders whose sand did not receive any admixture of nitrogenous compounds, we prepare others in which we further add to the sand 2·00 gr., 1·50 gr., 1·00 gr., 0·50 gr., or 0·10 gr. of Calcium nitrate.† In the course of the summer it is found that the oats unprovided with Calcium nitrate thrive at best poorly, while the power of elaborating material grows considerably with increase in the quantity of nitrate in the soil, and keeps pace with this increase. In the pea cultures on the contrary no exact relation is perceptible between the quantity of nitrate present and the capacity for growth, indeed it appears that the peas develop very vigorously even when nitrates are absent from the soil. By comparing the dry weight of the plants obtained with the dry weight of the seeds, we arrive at still more exact information

* A very suitable, double-washed Tertiary quartz sand from the Saxony Oberlausitz, which contains so little Nitrogen that it may be neglected, is to be obtained of the firm H. Weichelt & Co., Vereinigte Hohen-Bockaer Glassandgruben, Dresden.

† These quantities are not per kilogramme of sand, but per cylinder.

as to the growth capacity of the plants under the conditions described. If we estimate the Nitrogen in the dry substance (best by the well-known Kjeldahl method), we find among other things that the peas grown in nitrate-free soil often contain large quantities of Nitrogen, while the oat plants grown in nitrate-free soil are at most only a few milligrammes richer in Nitrogen than the seeds sown.

The peas (and other Papilionaceæ, *e.g.* lupins, beans, etc., behave in a similar manner), as we may already conclude from consideration of the results of these preliminary investigations, are able, in absence of nitrates from the soil, to utilise elementary Nitrogen for proteid formation. Oat plants and very many others cannot do this. Our present experiments, it is true, do not settle this latter point with absolute certainty; but we must pass by experiments which are thoroughly conclusive,[2] and proceed to investigate still further the behaviour of the Papilionaceæ towards elementary Nitrogen.

A number of glass cylinders of the dimensions above given are provided with quartz sand and food solutions, etc., but without addition of Calcium nitrate. The vessels are first carefully cleansed with sublimate-solution (1 : 1000), and then rinsed out with alcohol. The quartz and cotton wool are heated to 150° C. in the drying chamber before being introduced into the cylinders. The sand, mixed with Calcium carbonate in the quantity already specified, is heated in a large sand-bath for two to three hours to about 180° C., and filled into the cylinders while still warm. We now add to it the food solution, this having first been boiled for an hour in a flask closed with cotton wool, and then after two days for four hours in the steam steriliser * (to be obtained from H. Rohrbeck, Berlin. See Catalogue, 1887, p. 9). The seeds, peas, are first placed for two minutes in sublimate solution (1 : 1000), then rinsed with boiled water, and sown in the sand. The surface of the sand is covered with sterilised cotton wool. For watering nothing but boiled distilled water is to be used. We weigh the cylinders daily, and so determine the loss of water by evaporation, and hence how much water must be supplied to the soil.

One or more of the cylinders receive no further addition. To others are added 25 c.c. of an extract of fertile garden or field

* It might be better to put in the quartz and moist sand, mixed with food stuffs and Calcium carbonate, and then cover the cylinders with glass plates, and sterilise them in the steam of the steriliser.

soil, in which according to experience peas grow well. To prepare the extract we shake up 8 gr. of the soil with 100 c.c. of water, and allow to stand till the greater part of the sand and clay has settled, which often takes several hours. It must be observed that the quantity of combined Nitrogen in such an extract is so slight that it may be neglected in our investigation. Finally to the sand in a few cylinders is added soil-extract (again 25 c.c.), which has previously been sterilised by protracted boiling.

The culture vessels are placed in a room with a south aspect, the windows of which are kept closed. It is still better to conduct the cultures in a special small plant house which is not employed for the cultivation of other plants.

The experiments, if carefully made, show that all the seedlings at first grow normally. After three to four weeks, however, they manifestly get into a state of hunger. The plants in the cylinders without soil-extract and with sterilised extract do not recover, but henceforth gain a bare existence. Their dry weight at the end of the experiments, say after three to three and a half months, is very slight, and Nitrogen estimations show that they contain little or no more Nitrogen than the seeds sown.* The hunger stage is soon passed by the plants provided with unsterilised soil-extract. They thrive vigorously, develop ripe fruits and seeds (this takes place with peas in closed spaces from which insects are excluded), and produce large quantities of proteid.

Peas, and the Papilionaceæ in general, have therefore the exceedingly remarkable power of producing proteid in large quantities, although nitrogenous compounds are absent from the soil in which they are growing. In the Gramineæ, as also in many other plants, this capacity is entirely wanting; they thrive normally only when nutriment in the form of nitrogenous compounds stands at their disposal. The Papilionaceæ must be in a position to utilise free atmospheric Nitrogen for proteid formation, and this, as we shall see, can only come about through the intervention of bacteria.[3]

If shortly before the time of blooming, or during this period, we root up a pea plant (the observations may be made also with other Papilionaceæ) from the fertile soil in which it is growing, we shall

* Any increase in Nitrogen which may appear is due to the sand not being completely devoid of Nitrogen, and to the fact that the leave, as is known, can absorb some ammonia from the air.

find that its roots bear frequently a very considerable number of tubercles, sometimes of large size, which at this time are generally rose-red in colour. If we examine our pea plants grown in sand, we shall find that only the plants provided with unsterilised soil-extract have tubercles on their roots.

We now prepare median transverse sections of well-developed rose-red tubercles from pea roots, and examine first under lower and then under higher magnification. The middle of the section is composed of a very large-celled tissue, provided with intercellular spaces, whose cells are rich in plasma. In addition we perceive traversing this tissue, which is designated bacteroid tissue, characteristic branched highly glistening threads. Towards the outside the bacteroid tissue is bounded by a layer of parenchyma, which, on account of the starch contained in its elements, may be characterised as the starch layer. In this parenchyma run fibrovascular strands, which, as recent researches have taught, are in connection with the fibrovascular bundles of the roots bearing the tubercles. It is specially to be noted that the bast portions of the fibrovascular strands are directed inwards, *i.e.* towards the bacteroid tissue, while the wood is directed outwards. A superficial cortex is developed on the tubercles, which is composed of several layers of cuticularised cells.

If it is desired to study the developmental history of these tubercles in the Papilionaceæ, and the phenomena connected with their ultimate emptying, the student is referred for further information to Prazmowski's work (*Versuchsstationen*, Bd. 37, p. 209), since the space at our disposal does not permit us to enter here on this very complicated subject. It has been found that the highly refringent filamentous structures already mentioned as traversing the bacteroid tissue are tubes, which contain large numbers of bacteria, Bacterium Radicicola. This Schizomycete occurs in the soil, penetrates the root-hairs of Papilionaceous plants, here forms the tubes, which now grow into the cortex of the roots and induce the formation of tubercles. The tubes swell, and the bacteria present in them, continually and copiously multiplying, pass over into the bacteroid tissue of the developing tubercles, but after a time they lose their power of dividing, are metamorphosed, and form the so-called bacteroids, dichotomously branched corpuscles, which occur often in immense numbers in the cells of the bacteroid tissue.

Bacterium Radicicola, which stands in a symbiotic relationship

to the Papilionaceæ, is the organism which at the cost of elementary Nitrogen, clearly with the assistance of the carbohydrate supplied by the Papilionaceæ, produces proteid. When the tubercles empty, and the bacteroids disappear from their cells, the proteid passes into the organs of the plants, and is here employed in nutrition. If the soil in which, *e.g.*, peas are developing contains no Nitrogen compounds, then from what we have seen the development of the plants is only possible when the roots, by infection with Bacterium Radicicola, come to form tubercles, and for this reason only those of our objects exhibited considerable power of development which grew in unsterilised soil, or in sterilised soil watered with fresh soil-extract.

For the method of preparing pure cultures of Bacterium Radicicola, see Prazmowski, *Versuchsstationen*, Bd. 37, pp. 197 and 199, and also Beyerinck, *Botan. Zeitung*, 1888. Prazmowski (see p. 198) has also made investigations which teach that bacteria taken from pure cultures induce formation of tubercles on the roots of peas when the soil in which the objects are growing is infected with the fungus, while without such infection tubercle formation does not take place. Finally we find in Prazmowski (*Versuchsstationen*, Bd. 38, p. 20) directions for experiments, which by a very exact but also very complicated method enable us to prove that only Bacterium Radicicola, and no other form of Schizomycete, can bring about the nutrition of Papilionaceæ by means of elementary Nitrogen.*

In the course of Practical Plant Physiology which I conduct I include experiments intended for general information on the nutrition of plants with free Nitrogen, and proceed as follows:—Suitable glass cylinders capable of holding about 2·5 kilogrammes of sand are filled to a depth of 1–2 cm. with ignited quartz. To every 2·5 kg. of the sand referred to above are added in a large dish 375 c.c. of distilled water, 0·375 gr. H_2KPO_4, 0·200 gr. KCl, 0·200 gr. $MgSO_4$, 10 gr. $CaCO_3$, together with some phosphate of iron, and the mixture is crumbled into the cylinders. Each cylinder receives in addition 10 c.c. of soil-extract, prepared by treating 10 gr. of soil from a pea field with 100 c.c. of water. The quantity of Nitrogen compounds in such an extract is so slight that it may be neglected. Some cylinders

* It may further be remarked that we provisionally designate by the name Bact. Radic. not merely one, but a whole group of Schizomycetes, which are of importance in the nutrition of the Papilionaceæ.

receive each four peas germinated in sawdust, others four oat seedlings each. The cylinders are placed at a window looking to the south, and care is taken to replace daily the water lost by evaporation. The pea plants are supported by sticks. The peas having survived the starvation stage already referred to, thrive well, while the oats keep sickly. In cylinders, however, in which the sand receives an admixture of 0·250 gr. KNO_3 over and above the substances above specified, the oats flourish. The reasons for these differences in behaviour are found in what has gone before.

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[1] See Hellriegel, *Beilageheft zu der Zeitschrift d. Vereins f. Rübenzuckerindustrie d. Deutschen Reiches*, November, 1888.

[2] Such investigations are to be made according to the method of Boussingault (see *Agronomie*, i., p. 69). See also 19, above.

[3] Regarding the nitrogenous food of the Papilionaceæ, Gramineæ and other plants, I fully agree with the views of Hellriegel and Prazmowski (see *Versuchsstationen*, Bd. 37 and 38). Those of Frank, on the other hand (see *Landwirthschaftl. Jahrbücher*, and *Botan. Zeitung*, 1893), I cannot share; at all events the experiments of Frank are not thoroughly convincing.

21. The Detection of Ammonia and Nitric Acid in Water and in Plants. Nitromonas.

In order to satisfy ourselves that there occur in nature inorganic nitrogenous compounds (ammonia, Nitric acid) which can be utilised by plants in nutrition, it is well to test water (river, pond, or spring water) for both these substances. For ammonia we employ Nessler's solution. Two gr. of Potassium iodide are dissolved in 5 c.c. of water, the solution is heated, and to it we add rather more Mercuric iodide than it can dissolve. The solution after cooling is diluted with 20 c.c. of water, filtered and treated with potash solution (30 c.c. of potash solution, consisting 1 part of caustic potash to 2 parts of water, is added to 20 c.c. of the filtrate). We now fill two test-tubes with the water to be examined, treat with soda lye, filter if necessary, and add to the water in one tube about 30 drops of Nessler's reagent. A comparison of the tint of the fluids in the two tubes shows whether ammonia is present or absent, because in presence of ammonia the fluid becomes reddish in colour. To detect Nitric acid in water, a drop of the water is placed in a white porcelain dish, and treated with 2 drops of a solution of brucine (prepared by shaking up brucine in water). A few drops

of Sulphuric acid are now added.* A red coloration indicates the presence of Nitric acid in the water. To determine the presence of extremely small quantities of Nitric acid in water, we evaporate down a few c.c. of the water, and then treat the residue with brucine solution and Sulphuric acid.[1]

The presence of Nitric acid in water is also very conveniently determined by evaporating down a few c.c. of the water in a porcelain dish and adding to the residue, by means of a glass rod, diphenylamine dissolved in Sulphuric acid (0·05 gr. of diphenylamine dissolved in 10 c.c. of pure concentrated Sulphuric acid). A blue coloration is produced in presence of Nitric acid, owing to the formation of aniline blue.

Beetroot juice is fairly rich in nitrates. If we treat sections of the root on the slide with the diphenylamine solution, they become intensely blue.[2] A pretty lecture experiment for demonstrating the presence of nitrates in the root, is made by cutting a root in two, and simply applying diphenylamine solution to the cut surface. The blue coloration at once appears.

The occurrence of nitrates in plants has been exhaustively studied by Berthelot and André,[3] and by Schimper.[4] They found nitrates very widely distributed in plants, and if we cut sections of the stem of Helianthus, or *e.g.* leaves of Sambucus nigra, let them dry to some extent on the slide, and then treat them with a drop of the diphenylamine solution, the blue coloration instantaneously appears. In most leaves, *e.g.* those of Sambucus nigra, the nitrates are confined almost entirely to the nerve parenchyma; in some cases, however (leaves of Tradescantia Selloi), the mesophyll is also very rich in nitrates. Nitrites which also give a blue colouration with diphenylamine and Sulphuric acid, do not, as far as is at present known, occur in the living plant. It is to be emphasised that the failure of reaction with diphenylamine is not conclusive evidence of the absence of nitrates from the tissues under investigation, since many substances occur in plant cells whose presence would interfere with the reaction.

It is of great interest to establish the fact that there exist organisms which have the power of converting ammonia, occurring

* The Sulphuric acid must, of course, be free from Nitric acid, and not of itself give a red reaction with brucine solution. The acid may be freed from Nitric acid, which may be present, by boiling with a very small quantity of sulphur.

e.g. in the soil, into Nitric acid.[5] This nitrification is effected by a Schizomycete known as Nitromonas,* and there is perhaps no soil which is completely free from it.

We fill a flask with 100 c.c. of a culture fluid containing to every 100 c.c. of water 0·05 gr. $(NH_4)_2 SO_4$, 0·1 gr. $KH_2 PO_4$, and 1 gr. $Mg CO_3$. To the solution we add a little soil from arable land, so as to infect it with Nitromonas. After about a month, it can be proved by the diphenylamine reaction that a large quantity of Nitric acid has been formed in the fluid. The solution remains clear. A thin skin forms on the surface of it, and in the sediment of Magnesium carbonate are produced zoogloea forms of bacteria, through the mucilaginous nature of which the particles of carbonate are irregularly glued together. I observed this particularly well in a culture which had stood for three months.

A number of flasks are now provided with culture fluid as above. The flasks are closed with plugs of cotton wool, and the solutions are sterilised by boiling. After boiling for a long time we let the solutions cool, shake up the previously prepared Nitromonas culture, and infect each of them with a drop of it. One flask is left standing in the air; another is exposed to air free from Carbon dioxide, being placed in the apparatus described and figured in 16. After some time, *e.g.* a month, it is found that vigorous nitrification by the developing Nitromonas is only exhibited in the culture fluid to which atmospheric Carbon dioxide has had access. The Carbon dioxide of Magnesium carbonate cannot be worked up by this fungus. If culture fluid contained in a flask provided with a plug of cotton wool is sterilised, no formation of Nitric acid takes place in the solution if it has not been infected with Nitromonas germs. In all cases we must, of course, make sure that the culture fluid is free from nitrates at the commencement of the investigations. For this purpose we transfer a drop of the solution to a white dish, and add to it diphenylamine dissolved in Sulphuric acid.

Nitromonas belongs to a remarkable class of organisms which in the dark, and without chlorophyll, have the power of producing organic substance out of inorganic material. It is very difficult and troublesome to afford a strict proof of this assertion, and therefore we shall not pursue the subject further. It is especially

* Nitromonas is a bacterium, ellipsoid, and somewhat elongated in form, which does not produce filaments.

important to prepare first of all absolutely pure distilled water, and food stuffs which contain not a trace of organic material.

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[1] See Reichardt, *Grundlagen zur Beurtheilung des Trinkwassers*, 1880, pp. 154, 143.

[2] See Molisch, *Berichte d. Deutschen botan. Gesellschaft*, 1 Jahrg., p. 150.

[3] See Berthelot and André, *Annal. de chem. et de physique*, Ser. 6, T. 8.

[4] See Schimper, *Botan. Zeitung*, 1888, p. 120.

[5] Literature on Nitrification: see especially Schlösing and Müntz, *Compt. rend.*, T. 84, 85, and 86; Winogradzki, *Landwirthschaftl. Jahrbücher*, Bd. 20, p. 175; Godlewski, *Anzeiger der Akad. d. Wiss. in Krakau*, 1892.

22. Nitric Acid as a Nutrient Substance.

In the soil and in water occur nitrates which can be utilised as food by the higher plants. That the nitrates can actually furnish the Nitrogen needed for the production of proteids and the normal development of the plants, can be demonstrated by the water-culture method. Maize, oats, or buckwheat may be used for the experiments (I used maize with very good results). The experiments are carried out as described in 1. One or two plants are grown in a food solution, *a*, having the composition given in 1. Others are cultivated in a solution of similar composition, but containing, in place of Calcium nitrate, 1 gr. of Calcium sulphate per litre, *b*. The plants in *a* developed very normally in my experiments; those in *b*, on the other hand, presented after a few weeks a very distressed appearance. (See Fig. 22.) The lower leaves dried up, and the plants grew extremely slowly. They had at their disposal only the nitrogenous reserve materials of the seed. They could at most take up only small quantities of ammonia from the air, but these were not nearly sufficient for vigorous development.

23. Ammonia as Nutrient Material.

To convince ourselves that the higher plants can utilise for the formation of proteids, Ammonium salts absorbed by means of their roots, we grow maize or other plants in a food solution having exactly the same composition as that given in 1, but containing in place of $Ca(NO_3)_2$, 0·5 gr. of Ammonium phosphate, and 0·5 gr. of Calcium sulphate. Great care must be taken that the reaction of the solution does not change much during the experiments, but

is kept throughout slightly acid. If we attend to this point, the plants flourish well. The following solution may also be strongly recommended: 1000 c.c. of distilled water, 0·15 gr. Magnesium sulphate, 0·12 gr. Calcium chloride, 0·12 gr. Potassium sulphate,

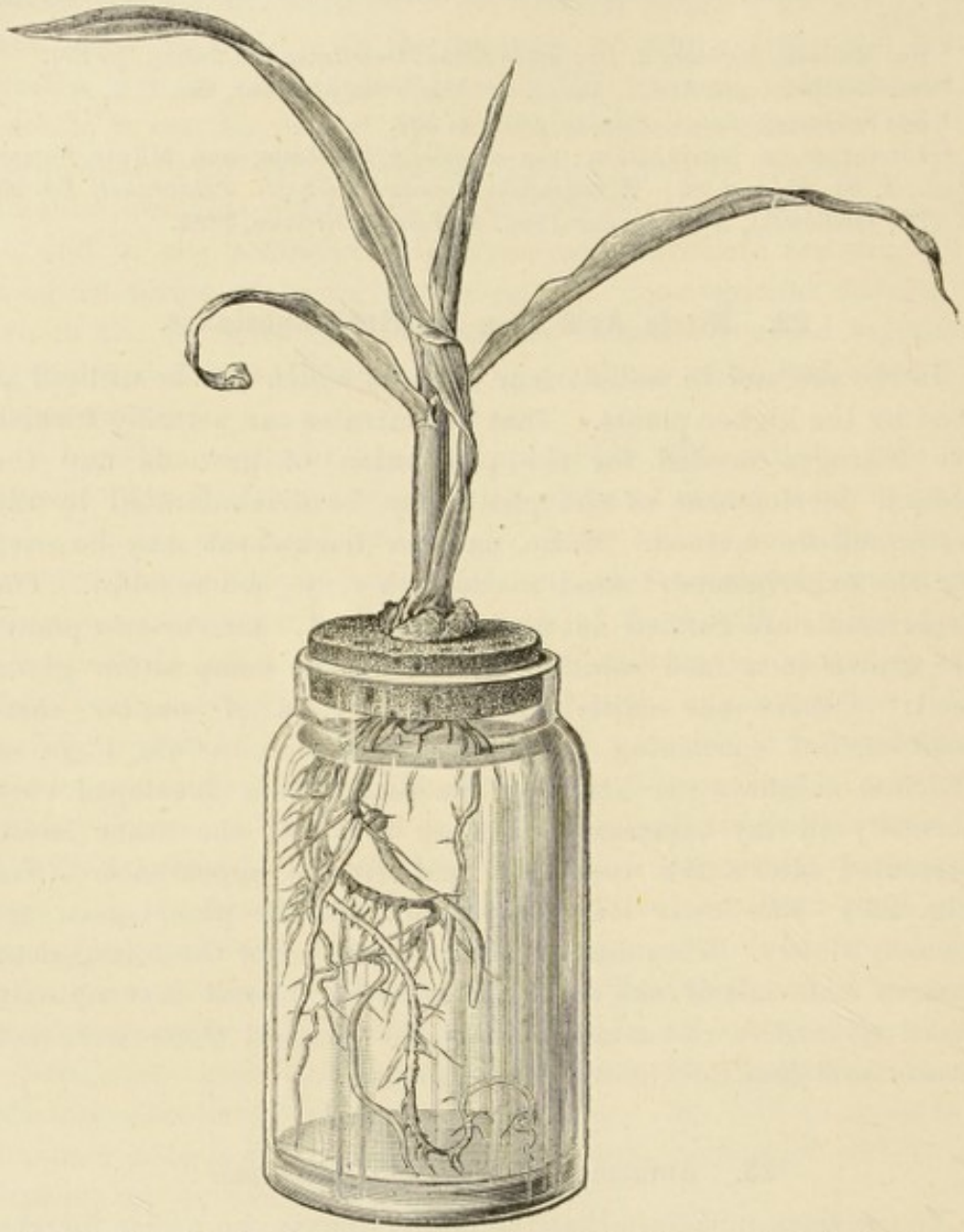

FIG. 22.—Maize Plant grown by the water-culture method in a nutrient solution devoid of nitrogenous compounds.

0·50 gr. acid Ammonium phosphate, and a very little Ferric chloride. Before using the solution, we add ammonia till its reaction is only slightly acid. The solution must be renewed frequently, say every five days. It is a question whether the

ammonia is not oxidised in the plant to Nitric acid, before being utilised for the formation of proteids. But still, the experiment teaches that ammonia can be employed by the higher plants as food.[1]

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[1] See G. Kühn and Hampe, *Versuchsstationen*, 1867.

24. The Seat of Proteid Formation in the Higher Plants.

In the leaves large quantities of carbohydrates are produced in assimilation. The transpiration current conveys to the leaves nitrates, and it may hence be assumed that the formation of proteids goes on chiefly in the leaves.

We prepare transverse sections through a mature leaflet of Trifolium pratense (see Fig. 3). Within the epidermis we see the single-layered palisade parenchyma, and the many-layered spongy parenchyma. The bast and wood of the vascular bundles are easily recognisable. The soft bast is enclosed on the outside by bast fibres, and the wood is similarly bounded on the outside by strongly thickened sclerenchymatous fibres. Outside the layer of bast fibres, and also outside the thick-walled cells encircling the wood, is a single layer of cells, devoid of chlorophyll, which contain crystals of Calcium oxalate. The crystal sheaths only cover the backs of the strands of fibres; they do not extend far laterally.[1]

Now since, as we shall see in 25, salts of Nitric acid (and also of Sulphuric acid) can be decomposed under the conditions obtaining in plants by the Oxalic acid which is so generally found in plant cells, it is not difficult to suppose that the crystals of Calcium oxalate referred to are to be regarded as the product of such reactions. The Nitric acid and Sulphuric acid can then, with non-nitrogenous organic material formed in the green cells, be employed under the influence of the protoplasm for the formation of proteids. With reference to these questions, the following observations and experiments claim particular attention.[2]

To prove the presence of Calcium oxalate in leaves (according to my experience it is very convenient, in order to learn the method, to use moderately young leaves of Humulus), we first kill the specimens under investigation by dipping in hot water, then extract them with alcohol, lay them in chloral hydrate solution (5 parts of water to 8 parts of chloral hydrate), and observe under the polarisation microscope. With crossed Nicols

the crystals of Calcium oxalate appear glistening white or coloured on a black ground; with parallel Nicols they appear almost black on a white ground. The crystals occur in the cells in very various forms. They belong partly to the tetragonal, and partly to the monoclinic system.[3] If we lay the objects in concentrated Hydrochloric acid, and again observe under the polarising microscope, we can easily make out the gradual solution of the oxalate. Water and Acetic acid do not dissolve the crystals.

Respecting the polarising microscope, consult Section II.

If we examine by the above method on the one hand young leaves, and on the other old leaves of Ampelopsis, we find in the parenchyma numerous bundles of raphides, and, in fact, in about equal quantities in both cases. Most Dicotyledons behave quite differently, and do not carry the Calcium oxalate in their leaves in the form of raphides. We examine *e. g.* young, just matured, and older leaves of Alnus glutinosa, Ulmus campestris, and Humulus. In all cases we take the leaves from shoots growing in the shade, and find that the quantity of oxalate in the parenchyma increases considerably with age.

If a vigorous pot plant of Pelargonium is put in a badly illuminated place, so that no assimilation can go on, then we find but little Calcium oxalate in the parenchyma of the newly originating leaves. The formation of these small quantities of oxalate is not associated with the action of light at all, and so we have here to do with primary Calcium oxalate. If the leaves develop in the dark, all formation of Calcium oxalate ceases. It begins again, however, if the plant is brought into the light. Secondary Calcium oxalate originates, whose production, unlike that of the primary oxalate, is dependent on the light. (Schimper.)

The material for the formation of the primary Calcium oxalate is afforded on the one hand by the Oxalic acid produced in the plant by metabolic processes, and on the other, by the lime absorbed by the roots from without. The latter enters the plant from the soil, chiefly in combination with Nitric acid and Sulphuric acid. If, now, the nitrates, for example, enter into chemical reaction with the Oxalic acid produced in metabolism, then, on the one hand, free Nitric acid is formed, which finds employment in proteid manufacture, and on the other hand, Calcium oxalate originates. Nitrates may also, however, transitorily accumulate in the plant tissue, as was shown by the observations mentioned in 21.

But to prove directly that nitrates can actually be worked up, we make the following experiment. Two pot plants of Pelargonium zonale are placed in the dark. The quantity of nitrates in the leaves of normally vegetating plants is generally very slight or nil. If after four to six days we examine the leaves of the darkened plants, it is found that they have become very rich in nitrates. If we now place the plants at the window, so that they are brightly illuminated, the nitrates disappear again from the leaves in the course of eight to fourteen days. If stems of Pelargonium zonale, or Fuchsia globosa, whose leaves are highly variegated, are treated as just described, we find that the nitrates do not disappear on illumination from the almost chlorophyll-free leaves.

Thus it follows, that the consumption of nitrates stands in relation to the chlorophyll of the leaves and to light. According to recent investigations, the working up of the Nitric acid and formation of proteid can proceed in the organisms of the higher plants only in the green cells, and under the influence of light.

According to Schimper (see also *Flora*, 1890, pp. 251 and 260), the working up of the nitrates, and of the sugar formed in assimilation into amides, and finally into proteid, organic acids (Oxalic acid) * perhaps being produced as bye-products, must be considered as quite directly dependent on light and chlorophyll activity. Proteid formation in chlorophyll-free cells, such as does actually take place in fungi is, according to him, excluded in the higher plants. In my opinion this view is not conclusively established. At the same time, the experiments by which many people seek to prove the formation of proteid in the higher plants, in cells which are not green, are inadequate.† The whole question needs further and very searching treatment, and it is possible that the light and the chlorophyll bodies are only indirectly of significance for proteid formation. The formation of the secondary oxalate also, might only indirectly be related to conditions of illumination.

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[1] See H. de Vries, *Landwirthsch. Jahrbücher*, Bd. 6, p. 900, and plate 44, Fig. 3.

[2] See Schimper, *Botan. Zeitung*, 1888.

[3] For illustrations of the Calcium oxalate crystals, see Zimmermann, *Botan. Microtechnik*, 1892, p. 56. (Trans. Humphrey.)

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* This Oxalic acid may then give rise to secondary Calcium oxalate.

† Frank (*Lehrbuch d. Botanik*, 1892, pp. 568 and 570) holds the view that perhaps proteid formation is possible in the higher plants in all the cells.

25. The Decomposition of Nitrates in Plants.

It has already been pointed out (see 24) that the Nitric acid necessary for the formation of proteid is liberated from nitrates by the action of vegetable acids, especially Oxalic acid, with simultaneous production of oxalates. This and similar reactions are further of importance because they serve to free the plant sap from excess of Calcium salts, Calcium oxalate being of course only very slightly soluble, and, in fact, very generally separating out in plants in the crystalline form.

The interaction between Oxalic acid and lime or potash salts in the plant must take place in very dilute solution, and hence it is interesting to ascertain by experiment whether the reactions in question do proceed in presence of very large quantities of water.[1]

We get ready a number of beakers containing solutions of 0·205 gr. of Calcium nitrate in 400 c.c. of water. We further prepare solutions containing 0·090 gr. of Oxalic acid (considered anhydrous) to every 100 c.c. of water. If now we mix these solutions together in pairs, 400 c.c. of Calcium nitrate solution to 100 c.c. of Oxalic acid solution, at the ordinary temperature, precipitates of Calcium oxalate are formed and Nitric acid is set free. Time, however, is a factor in determining the extent of the reaction, as we may learn by ascertaining the amount of precipitated Calcium oxalate in one case immediately, in others after the lapse of one, two, or three days. The longer the Oxalic acid is allowed to act on the Calcium salt, the greater the quantity of Calcium oxalate precipitated.

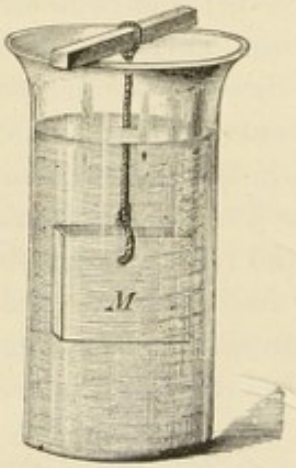

Fig. 23.—Apparatus for investigating the action of Oxalic acid on nitrates.

That Nitric acid is actually liberated when Oxalic acid acts on Potassium nitrate we can prove as follows:—Five beakers, *a*, *b*, *c*, *d*, and *e*, are arranged each containing 500 c.c. of water. To *a* we add 0·210 gr. of Nitric acid, HNO_3; to *b*, 3·000 gr. of Oxalic acid (considered anhydrous); to *c*, 0·337 gr. of Potassium nitrate; to *d*, 0·210 gr. of Nitric acid and 3·000 gr. of Oxalic acid; to *e*, 0·337 gr. of Potassium nitrate and 3·000 gr. of Oxalic acid. In each beaker is suspended a slab of marble (see Fig. 23, *M*), all the slabs being as nearly as possible of the same size, say

40 mm. long and broad and 5 mm. thick. They are supported by threads fastened to sticks which rest on the rims of the beakers. If the preparations are allowed to stand for some time, it is found that the solution in *a* does not become turbid; neither does that in *b*, since the marble becomes covered with a crust of Calcium oxalate, which arrests the further action of the Oxalic acid. The fluid in *c* also remains clear, but in *d* and *e* considerable precipitates of Calcium oxalate are thrown down. In *d* the Nitric acid acts on the marble. It gives rise to Calcium nitrate, which now is decomposed by the Oxalic acid present, with formation of Calcium oxalate and liberation of Nitric acid. This latter then acts again on the marble. If, as is actually the case, a considerable precipitate is produced in *e*, it can only be explained as due to the liberation of Nitric acid by the action of the Oxalic acid on the Potassium nitrate present in the solution, this Nitric acid then behaving like that in *d*. These reactions in *d* and *e* proceed so rapidly, as I have convinced myself, that even in the course of half an hour a considerable amount of Calcium oxalate separates out. We can therefore in the above manner demonstrate quite conveniently even in lecture the fact that Oxalic acid is able to decompose Potassium nitrate.

[1] For further information respecting nitrate decompositions consult Emmerling, *Versuchsstationen*, Bd. 17 and 30.

III. THE CONSTITUENTS OF THE ASH OF PLANTS

26. Mechanical Analysis of Soil.

In judging of the character of a soil, it is of great importance to ascertain exactly the amount of coarser and finer elements in it. We therefore distinguish, as has long been customary, between the skeleton and the fine earth of the soil. The latter demands some special attention, since it determines the most important characteristics of the soil, and chiefly furnishes the plant with the mineral matter which it requires for its nutrition. To obtain fine earth for the purpose of chemical analysis, air-dry soil is riddled through a sieve with meshes 0·5 mm. wide. But if it is desired to determine the quantity of fine earth contained in a soil, we treat 50 gr. of air-dry earth in a dish with water, heat on the water-bath for about an hour, and then transfer to a sieve with 0·5 mm. meshes, the fine earth being washed through, first with the help of a paint brush and finally by means of a fine

stream of water. The residue on the sieve (skeleton) we dry and weigh. We may further subdivide the skeleton into writing sand (*Streusand*), fine gravel (*Feinkies*), medium gravel, and coarse gravel. This is likewise effected by means of sieves. If we work with Knop's series of sieves, the term coarse gravel is applied to the residue left on a sieve with meshes 4·2 mm. wide; medium gravel to that left in a sieve with 2·7 mm. meshes, and fine gravel to that left in a sieve of 0·9 mm. meshes.

The fine earth is divided by washing into fine sand and clayey constituents. The apparatus required is Kühne's washing cylinder (Fig. 24). The glass cylinder is 28 cm. in height, and 8·5 cm. in diameter (both inside measurements). At a distance of 5 cm. from the base of the cylinder is a tubulure which can be closed in the manner indicated in the figure. We place in the cylinder 30 gr. of fine earth which has previously been heated for some time with water, fill the cylinder with water up to the level of a mark towards the top, stir with a rod, and then let stand for ten minutes. The turbid fluid is now run off, and we pour a fresh quantity of water into the cylinder, stir up, and after five minutes again remove the fluid. We proceed in this way until all the clayey part appears to be washed away, then dry the residue (fine sand) which remains behind in the cylinder, and determine its weight.[1] The fine earth consists, as such analyses among other things teach, of granules of very different sizes, a fact of which we may at once satisfy ourselves by mounting some of the fine earth in water on a slide, and submitting it to microscopic examination. The washing cylinder mentioned, and also sieves, are to be obtained of Muencke, in Berlin.

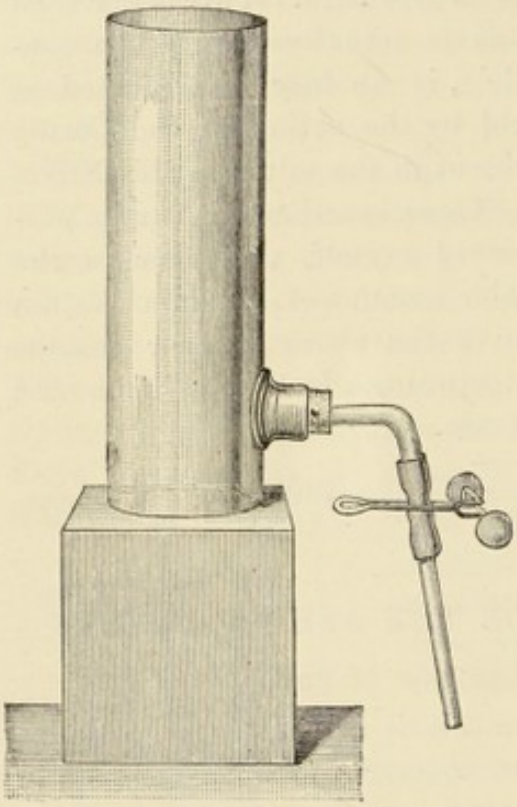

Fig. 24.—Washing cylinder.

[1] A full discussion of the function of the *fine earth* and *skeleton*, and of the

method of mechanical analysis of the soil will be found in my *Lehrbuch der Bodenkunde*, 1876, and in my treatise on the soil which appears in Bd. 2 of the *Handbuch der Landwirthschaft*, 1888, edited by Goltz.

27. The Detection of Certain Food Stuffs in the Soil.

It is here by no means my intention to give a detailed introduction to the chemical examination of the soil.

Any one wishing to take up such researches, and intending to make himself exactly acquainted with the value of the results that have been obtained must be referred to the works of E. von Wolff,[1] Knop,[2] and to my *Bodenkunde*.[3] It is merely intended here to prove that the soil contains certain substances which serve as food for plants. For the experiments we use always the fine earth of the soil, isolated in the manner described in 26.

To prove the presence of Chlorine in the soil, we pour 200 c.c. of water over 5 gr. of fine earth and allow to stand for some time. We then filter and determine the Chlorine in the filtrate in the usual way by means of Silver nitrate.

To determine the presence of Sulphuric acid in the soil, 2–5 gr. of fine earth are mixed with 20-50 gr. of Sodium carbonate free from Sulphuric acid. The mixture is placed in a porcelain dish, treated with water and boiled for some time. In the fluid obtained, Sulphuric acid can readily be detected qualitatively or quantitatively estimated.

About 10 gr. of fine earth are mixed with water, and then with constant stirring treated with Hydrochloric acid until the evolution of Carbon dioxide has ceased. After some time we filter off the slightly acid solution, nearly neutralise with ammonia, and warm gently with Sodium acetate. A precipitate is formed, consisting chiefly of Ferric oxide, while in the fluid filtered away from the precipitate we can readily detect by means of Ammonium oxalate the presence of Calcium.

We shall not here consider the somewhat troublesome processes necessary for determining the presence in the soil of other substances, especially of Potassium and Phosphoric acid. As regards these the works cited above must be consulted.[4]

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[1] See E. von Wolff, *Anleitung zur chem. Untersuchung landwirthschl. wichtiger Stoffe*, 1875.

[2] Knop, *Die Bonitirung der Ackererde*, 1871.

[3] See Detmer, *Lehrbuch der Bodenkunde*, 1876.

[4] See also König, *Untersuchung landwirthschl. wichtiger Stoffe*, 1891.

28. Food Stuffs in Water.

It is of interest to determine the presence in water of some substances which are of importance as plant food, taking for examination, river, pond, or spring water. We acidify 20 c.c. of water with a few drops of Hydrochloric acid, add Barium chloride solution; turbidity or precipitate indicates the presence of sulphate. Acidify 20 c.c. of the water with pure HNO_3 and add Silver nitrate solution; a white curdy precipitate indicates the presence of chloride. 50 c.c. of the water are acidified with Hydrochloric acid, excess of ammonia is added and lastly Ammonium oxalate; a white precipitate indicates the presence of Calcium. Lime occurs in water in combination with Sulphuric acid or with Carbonic acid (Calcium bicarbonate). The presence of this latter compound is detected by heating the fresh water, since it is easily decomposed on heating into the normal Calcium carbonate, which separates out, rendering the fluid turbid, and Carbon dioxide. If spring water, fairly rich in Calcium bicarbonate, is laid aside for some time in a small glass at the ordinary temperature, crystals of normal Calcium carbonate separate out, whose form we can easily make out by microscopic examination.

Since we do not here propose to give an introduction to the examination of water, but only to establish the fact that water contains food stuffs, we will now leave the subject. It has, however, already been indicated in another place how to proceed in examining water for ammonia and Nitric acid.[1]

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[1] For an exact account of the method of water examination, see Tiemann, *Anleitung zur Untersuchung von Wasser*, 1874, and Reichardt, *Grundlagen zur Beurtheilung des Trinkwassers*, 1880.

29. Ash Analysis.

Although qualitative and quantitative ash analyses are somewhat troublesome and tedious, I strongly advise any one who intends to occupy himself with physiological studies to carry out researches of this kind. The plant material is first carefully cleansed, being freed, *e.g.* from adhering dust. Fresh roots must then be cut up into slices, which, as also fresh stems and leaves, are hung up in the drying chamber at a temperature of about 50° C. to dry. The dried roots are pounded up to a moderately fine powder; the dried stems or leaves are cut up with scissors. Air-dry seeds are to be pounded to a coarse powder in a mortar.

Suppose it is desired to investigate the ash of the aerial parts

of clover plants. A large quantity of the clover is dried in the drying chamber and cut up, the whole being then thoroughly mixed together. About 100 gr. of the material will yield sufficient ash for our purpose. The combustion is best effected in a large platinum dish; in absence of this a porcelain dish may be used. In the combustion itself, which is effected over an open flame, great care must be taken that the temperature does not rise too high; the ash should never be allowed to glow. The analysis is conducted according to the directions of E. von Wolff.[1] I have often convinced myself that this method is satisfactory. 1 gr. of the crude ash obtained serves for the determination of Carbon dioxide, which may conveniently be carried out by means of Dietrich's apparatus.[2] 1 gr. of crude ash is also treated with diluted Nitric acid, the solution serving for the determination of Chlorine. 3–4 gr. of ash is moistened in a flask with concentrated Nitric acid and then treated with strong Hydrochloric acid, and digested for some time at boiling point. The whole is now rinsed into a porcelain dish, and evaporated to dryness. The dry residue is placed for some time in the drying chamber, then moistened with concentrated Hydrochloric acid and extracted with water. The undissolved residue (Silicic acid, sand, Carbon) is collected on a dried and weighed filter, well washed with hot water, dried and then weighed. The contents of the filter are transferred to a platinum dish, and, after addition of soda lye, boiled several times with a concentrated solution of Sodium carbonate. The fluid is now filtered through the filter paper already used. The residue after being well washed consists of sand and Carbon, the quantity of each being determined by combustion. The alkaline solution serves for the determination of the Silicic acid. It is treated with excess of Hydrochloric acid, and evaporated to dryness, the residue being then boiled with acidified water. Silicic acid is separated and its weight is determined.

The filtrate from the above-mentioned residue of Silicic acid, sand, and Carbon is made up to a known volume, say 500 c.c., and divided into two measured portions. In one portion we determine the Sulphuric acid by means of Barium chloride. The solution separated from the Barium sulphate by filtration is warmed gently with ammonia, Ammonium carbonate and Ammonium oxalate, and filtered. The filtrate is evaporated to dryness, the residue gently ignited and heated with Oxalic acid. The ignited residue must be treated with water. The solution

obtained is filtered and treated with Hydrochloric acid, and then again evaporated to dryness. The residue is slightly ignited, and we determine the weight of the now pure alkaline chloride left. The Potassium is separated from the Sodium in the usual manner by means of Platinic chloride. The second portion of the solution must be nearly saturated with ammonia, treated with Ammonium acetate, and gently warmed in order to precipitate the Ferric phosphate present. The Phosphoric acid and the Ferric oxide are then estimated according to the formula $Fe_2(PO_4)_2$. To the filtrate from the Ferric phosphate we add Ammonium oxalate, and heat till it begins to boil in order to separate out the Calcium (lime determination). After evaporating down to about 150 c.c., the filtrate is treated with large excess of ammonia. After 24 hours the precipitated Ammonium Magnesium phosphate is collected on a filter, and we estimate the quantity of Phosphoric acid and magnesia in it. The filtrate will give a precipitate with magnesia mixture (prepared by mixing 1 part of crystallised Magnesium sulphate with 2 parts of Ammonium chloride, 8 parts of water, and 4 parts of ammonia) if it still contains Phosphoric acid, or with Sodium phosphate if magnesia is still present.

In tabulating the results of quantitative analyses of ash, the amount of crude ash present in the dry substance must be given as well as the quantity of pure ash (*i.e.* crude ash less Carbonic acid, sand, and Carbon). The percentage composition of the pure ash must also be calculated.

The ash of some plants, *e.g.* that of the tobacco plant, contains also Lithium. We can easily prepare a pretty lecture experiment by moistening cigar ash with dilute Nitric acid, and holding a little of the mixture in the non-luminous flame of a Bunsen burner or spirit lamp on a loop of platinum wire fused into a glass rod. Spectroscopic examination of the flame, *e.g.* by means of a small pocket spectroscope, almost always discloses at once the very characteristic red Lithium line.

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[1] See E. von Wolff, *Anleitung zur chem. Untersuchung landwirthschl. wichtiger Stoffe*, 1875, p. 159.

[2] See Dietrich, *Zeitschrift f. analytische Chemie*, Bd. 3 and 4. The Dietrich apparatus can be obtained from J. H. Büchler, Breslau.

30. The Higher Plants Need to be Supplied with Mineral Substances. Sodium and Silicon are not Essential.

The problems here under consideration can be studied most conveniently and accurately by the water culture method. The

mode of procedure has been dealt with at length in 1, and we grow maize plants according to the instructions there given in a complete food solution, one therefore which contains Calcium nitrate, Potassium chloride, Magnesium sulphate, Potassium phosphate, and some Ferric chloride. But simultaneously we also attempt to bring to maturity a maize plant, germinated in sawdust, whose roots are growing merely in distilled water. This latter plant soon ceases to grow, while those whose roots are growing in a complete food solution continue to develop vigorously. In my experiments, the maize plants with distilled water at their disposal unfolded only four leaves; their growth then ceased. Mineral substances are thus necessary for normal development. If the roots of plants are surrounded by distilled water and not by a food solution, their growth comes to a standstill when the reserve materials of the seed are exhausted.

Our food solution is free from soda and silica. The experiment therefore teaches further that neither Sodium nor Silicon is to be considered as an essential constituent of the food. It is, however, possible that for some organisms Silicon is necessary, as *e.g.* in the case of Diatoms, the microscopical brown organisms so often met with on plants or stones in water, and whose membranes are impregnated with large quantities of silica. In the case of grasses silica may perhaps be spared, but at the same time it is useful, and this may also be the case with the Equisetums. At any rate the amount of silica in the walls of the epidermal cells of the latter is very considerable, as we may satisfy ourselves as follows.

If we examine surface sections of the stem of Equisetum arvense we find that strips bearing stomata and strips devoid of stomata alternate with each other. The former, owing to the layer of chlorophyll-containing cells below them, appear green; the latter colourless. The epidermal cells are longitudinally elongated; the structure of the stomatal apparatus is a complicated one. We lay a surface section on a small plate of mica, treat the section with concentrated Sulphuric acid, and strongly heat it in a gas or spirit flame. We then lay the mica plate, with the ash, on a slide, add a drop of water, and cover the preparation with a cover glass. On microscopic examination we perceive a silicious skeleton which still allows much of the essential structure of the original section to be made out.

31. Phosphorus, Sulphur, Potassium, Calcium, Magnesium, and Iron, are Necessary for the Higher Plants.

We cultivate plants, *e.g.* maize plants, by the water-culture method, as described in 1, but using a complete food solution of somewhat different composition. It contains per litre, in addition to a very small quantity of Ferric chloride, 1 gr. of Calcium nitrate, 0·5 gr. of Potassium chloride, 0·5 gr. of Magnesium sulphate, and 0·5 gr. of finely powdered Calcium phosphate ($Ca_3 (PO_4)_2$). This last is very sparingly soluble in water, and therefore forms a sediment in the culture vessel. We further prepare a solution devoid of potash, the Potassium chloride of the complete food solution being replaced by 0·5 gr. of Sodium chloride. We make a third solution free from Phosphoric acid, the Calcium phosphate being replaced by 0·5 gr. of Calcium sulphate. A solution free from iron is obtained by leaving out the Ferric chloride. The culture vessels, after they have been fitted up with maize plants, are all exposed to the same external conditions. It is found that the plant in the complete food solution thrives extremely well, while those in the solutions wanting in potash and Phosphoric acid respectively soon cease to grow, when, viz., the small quantities of these substances stored in the seed are exhausted. Potassium and Phosphorus are therefore absolutely essential. The culture in the solution free from potash teaches still further that Potassium cannot in higher plants be replaced in nutrition by Sodium, which chemically is so nearly related to it. Solutions devoid of sulphur are also easily prepared.

Plants grown in solutions devoid of iron produce at first normal green leaves. Very soon, however, they begin to appear sickly; in fact, when the iron in the seed has been used up, they become icteric and chlorotic. The new leaves are no longer green, but white, and microscopic examination of them shows that abnormal chlorophyll bodies, or none at all, are present in their cells. If we add to the food solution a few drops of dilute Ferric chloride solution, the previously white leaves become green in two or three days, and the growth of the plants now proceeds normally.[1]

We may also conveniently work with the following food solutions. We shall find that in the normal solution our plants flourish well, but that with solutions wanting in one substance or another they soon die.

All the solutions except the last one, containing no Phosphoric

acid, are to be diluted before use with distilled water, in the proportion of 1 : 4·8.[2]

Normal Solution :*—

600 c.c. distilled water,
7 gr. Potassium nitrate,
1·5 gr. Magnesium sulphate,
1·5 gr. Sodium chloride,
1·5 gr. neutral Potassium phosphate.
Gypsum ($Ca\ SO_4$) in excess.

Solution without Lime :—
The above *minus* Gypsum.

Solution without Potash :—

600 c.c. distilled water,
7 gr. Calcium nitrate,
1·5 gr. Magnesium sulphate,
1·5 gr. Sodium chloride,
1·5 gr. neutral Sodium phosphate.

Solution without Magnesia :—

600 c.c. distilled water,
6 gr. Calcium nitrate,
1·5 gr. Potassium nitrate,
1·5 gr. neutral Potassium phosphate,
1·2 gr. Potassium sulphate.

Solution without Phosphoric acid :—

1000 c.c. distilled water,
0·5 gr. Potassium nitrate,
1 gr. Calcium nitrate,
0·5 gr. Magnesium nitrate,
0·5 gr. neutral Potassium sulphate.

That plants, *e.g.* maize and beans, which we may also employ for experiment with the above solutions, soon die in solutions free from Phosphoric acid, is quite natural when we consider that Phosphorus must be regarded as an essential constituent of the nuclein, which appears to be indispensable for the formation of nuclei.

If plants are cultivated in solutions containing no lime, they grow at first normally. Later on, however, they show signs of

* To all the solutions, dilute Ferric chloride solution must be added.

sickness, since particularly the youngest, and later also the older leaves, become spotted with brown. The leaves obtained have about the same size, and also contain the same quantity of chlorophyll as those formed under normal conditions. They can assimilate, like these latter, but still ultimately die off. The leaves of plants grown without lime differ from normal leaves in being very rich in starch. The brown stains which after a certain time appear on them, are, as the investigations of Schimper and Loew seem to show conclusively, due to action on the tissues of injurious organic acids, especially Oxalic acid. Organic acids are of course abundantly formed in plant metabolism. They must be neutralised, and the function of the lime is especially to effect this. If the neutralisation of the acids is not affected, some processes in the cells are arrested, and finally the cells completely die off. Lime is also of importance in the nutrition of plants, inasmuch as inorganic acids (Nitric and Sulphuric) are introduced into the plant chiefly in combination with lime.

Potash appears to play an important part in the origin and development of plant organs. If we cultivate plants in solutions devoid of potash, their behaviour is quite different to that in absence of lime. At first the Potassium compounds migrating from the older parts still serve to a certain extent to satisfy the requirements of the new organs. We shall, however, observe that these organs, *e.g.* the leaves, become smaller and smaller, till finally the plant dies. In the plants raised without potash compounds, the assimilatory activity also gradually ceases (Nobbe, Schimper), but still not till other indications of ill-health are already apparent, especially the limited growth of the new organs.

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[1] I have collected the literature on the subject in my *Lehrbuch der Pflanzenphysiologie.*

[2] See Schimper, *Flora*, 1890, Heft 3.

32. The Requirements of Fungi.

The development of fungi, like that of higher plants, is dependent on the presence of mineral substances capable of absorption. To prove that this is the case we make appropriate culture experiments with the yeast fungus (Saccharomyces cerevisiæ). We prepare a good quantity of a fluid having the following percentage composition:—84 parts of water, 15 parts of the purest sugar candy, and 1 part of Ammonium tartrate. Four flasks are

obtained, and into each is poured 100 c.c. of the fluid. To *a* we make no addition; to *b* we add 0·2 parts of acid Potassium phosphate ($KH_2 PO_4$), 0·02 parts of Calcium phosphate ($Ca_3 2 PO_4$) and 0·02 parts of Magnesium sulphate ($Mg SO_4$); to *c* we add 0·02 parts of Calcium phosphate and 0·02 parts of Magnesium sulphate; and, finally, to *d* are added 0·2 parts of Sodium phosphate ($Na_2 HPO_4$), 0·02 parts of Calcium phosphate, and 0·02 parts of Magnesium sulphate. Regarding addition of Ferric salt, see p. 58. We now close the flasks with stoppers of cotton wool, heat the fluids to boiling, and allow to cool again. We next add to each of the four nutrient solutions 1 c.c. of slightly milky yeast water (see Appendix), momentarily raising for this purpose the plugs of cotton wool with which the flasks are closed. It is best now to put the four flasks into the thermostat at a temperature of 25° to 30° C., frequently shaking them. Vigorous fermentation and multiplication of the yeast only takes place in the fluid *b*, as we may quantitatively determine by collecting the yeast on a dried and weighed filter. If a slight turbidity appears in the fluids *a*, *c*, and *d* (due to a small development of yeast or bacteria), that must be taken as an indication of the presence of small quantities of mineral substances or potash, as the case may be, which were perhaps present in the sugar, or in the yeast water added to the fluids. At all events, the yeast cannot develop normally when mineral substances are wanting in the food solution. But our experiments teach further that no extensive vegetation of the yeast and no fermentation takes place even when only Potassium is absent,[1] this not being replaceable by Sodium. In the experiments the greatest care must be taken as to the purity of the water, of the reagents, and of the yeast material. The more favourable the conditions in this direction, the more striking will be the result of the experiments.

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[1] For further information as to the food requirements of yeast, see A. Mayer, *Lehrbuch der Gahrungschemie*, 1874, p. 121.

33. The Forms in which Certain Mineral Substances Occur in Plants.

Mineral substances are by no means restricted in the plant to inorganic compounds; they also occur very frequently in organic substances also, *e.g.* Sulphur in proteids, Phosphorus in

nuclein, etc. This is of great importance, especially if we take into consideration the further fact that during the development of the plant any particular substance may be transferred from an organic to an inorganic compound, and *vice versa*. The study of these relations, which is of importance in considering the function of mineral substances, and in other respects, has lately been stimulated especially by Schimper,[1] and we will first of all make ourselves acquainted with the method of examination, confining ourselves to the detection of Phosphoric acid, potash, and lime. Further details must be sought in Schimper and Zimmermann.[2]

To detect mineral substances microchemically, sections, not too thin, must first be treated directly with reagents, while others are tested after being reduced to ash by ignition on the slide. Organic mineral compounds frequently cannot be found in the unaltered sections; generally the presence of bases or acids can then only be made out easily in the ash. The purity of the reagents to be employed must be very carefully tested.

To detect Calcium in the ash from the section, we dissolve it on the slide in 2 per cent. Sulphuric acid, and allow to dry up slowly. Then, particularly at the edges of the drop, characteristic crystals of Calcium sulphate are formed. See drawings in Haushofer, *Microskopische Reactionen*, Brunswick, 1885, and Zimmermann's work, cited below, p. 64.

The detection of Potassium is effected with Platinic chloride. The reagent must be specially tested as to its purity. The crystals of double chloride of Potassium and Platinum which are produced, are in the form of regular octahedrons or cubes. The sections are treated with a drop of the reagent, and allowed to dry up. The ash is dissolved in a little acidified water, the object glass is held over a spirit flame till the solution dries up, and then the reagent is added. The crystals always form first at the edge of the drop; later they appear in the middle also.

Phosphoric acid we isolate microchemically as Ammonium Magnesium phosphate. The section, or the ash of it (the latter after solution in acidified water and evaporation of the solution on the slide), is treated with a small quantity of a mixture of 25 vols. of a concentrated aqueous solution of Magnesium sulphate, 2 vols. of concentrated aqueous solution of Ammonium chloride, and 15 vols. of water. The originating crystals of

Ammonium Magnesium phosphate (in examining sections they are produced in the cells) have very characteristic forms.

Very searching investigation is still required as to the distribution of organic and inorganic compounds of mineral substances. But still some results have been obtained.

In resting seeds (we examine sections from soaked seeds of Phaseolus and Ricinus), Phosphoric acid, lime, and potash occur in organic compounds, and so are detected not directly in the sections, but in the ash which these yield. In germination these organic compounds are split up. Phosphoric acid, lime, and potash travel as inorganic salts in the parenchyma of the cortex and pith, both of stem and root, and also in the parenchyma of the leaf nerves. In all these tissues the above substances can therefore be found directly (not merely after incineration). For example, the parenchyma of maize seedlings is rich in potash and phosphoric acid.

In the growing points and in the mesophyll of seedlings occur large quantities of organic Phosphorus compounds, while inorganic compounds of Phosphorus are entirely wanting. The Phosphoric acid can hence be detected only by examination of the ash. The growing points and the mesophyll are thus the limits of migration, places of formation of Phosphorus-containing organic bodies. The parenchyma of cortex, pith, and leaf-nerves, on the other hand, forms the channels in which the mineral substances travel during germination.

It may be remarked further that the growing points, the cambium, and the sieve tubes (we may *e.g.* test the sap of the sieve tubes of Cucurbita, and also the ash which it yields) of seedlings and grown plants are almost or entirely devoid of lime, while they contain more or less large quantities of potash and Phosphoric acid, chiefly in the form of organic compounds.

To familiarise ourselves with the method of testing microchemically for iron, we soak seeds of Sinapis alba in water, then remove the seed-coat, and lay the embryo, which, as is well known, bears folded cotyledons, in a 2 per cent. solution of yellow prussiate of potash (Potassium ferrocyanide). After half an hour we transfer it to 20 per cent. Hydrochloric acid, wash after some time with distilled water, and spread out the cotyledons on the slide.* Only the primordia of the vascular bundles of the cotyledons (and

* Steel needles naturally must not be used.

in the case of root its central strand) have stained blue, and stand out as a blue network.

In the germination of the mustard seed, whether in light or darkness, the iron, in the form in which it was present in the resting seed, completely disappears from the objects after some time. It doubtless passes into organic compounds, in which it can no longer be detected by the methods indicated above.[3]

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[1] See Schimper, *Flora*, 1890, Heft 3.

[2] See Zimmermann, *Botan. Mikrotechnik*, 1892, p. 46. (Trans. Humphrey.)

[3] See Molisch, *Die Pflanze und ihre Beziehungen zum Eisen*, Jena, 1892. The method recommended in this paper by Molisch for the detection of iron present in organic compounds or disguised, is defective, as Molisch himself and C. Müller (both in *Berichte d. Deutschen botan. Gesellschaft*, Bd. 11) have shown.

IV. ORGANIC COMPOUNDS AS FOOD FOR PLANTS.

34. Humus. Mycorhiza.

In the soil are present a whole series of different organic compounds which owe their origin to the change and decay of vegetable and animal remains. These we may term humous compounds. It is not impossible that some green plants (*e.g.* particular marsh plants) can satisfy their requirements as regards organic substances, in part at least, at the expense of humous material, and certainly humous compounds play an important part in the nutrition of many fungi (Agarici and Boleti, etc.). We must therefore familiarise ourselves with the humous substances of the soil. Humous compounds are present in almost every soil, though, it is true, in very different quantities. Turf soil is particularly rich in humus, and for this reason we select it for examination.

We break up some turf in water in a porcelain dish, and add potash solution to the mixture. The fluid takes on a brown or black coloration. It contains Potassium humate in solution, and may be filtered off from the insoluble residue of the turf. We shall not further consider this residue; it contains a mixture of not yet completely humified plant remains, humic acid not yet extracted by the potash, and a substance (humin) insoluble in potash. We add Hydrochloric acid to the solution of Potassium humate until the fluid is distinctly acid in reaction. The Humic acid is thus precipitated, while the acid fluid still

retains in solution small quantities of certain humous substances. If we collect the Humic acid on a filter, wash well with water, and dry, we get a brittle, crumbling black mass, which is almost insoluble in water. If, on the other hand, still moist Humic acid is treated with water, somewhat larger quantities of the substance pass into solution. This solution of Humic acid has a yellowish brown colour. If we add Humic acid to solution of ammonia, it readily dissolves with formation of Ammonium humate. If we add to this solution a solution of Calcium chloride, a precipitate is produced consisting of the double humate of Calcium and Ammonium, a compound which can also undoubtedly be formed under some conditions in nature. The soil certainly contains in addition a whole series of other humous compounds, about which, however, nothing is accurately known.

FIG. 25.—Beech root grown in unsterilised wood humus: *p*, strands of fungal hyphæ, at *a* associated with humus. Magnified several times. (After Frank.)

FIG. 26.—Beech root grown in wood humus freed from fungus by sterilisation. It is not provided with fungal hyphæ, and has root hairs *h*. At *c* is the root tip with its root cap. Several times magnified. (After Frank.)

We carefully pull up from the humous soil of a wood a young beech plant (Fagus sylvatica), and convey it, wrapped in moist moss, into the laboratory. After washing the root with water, we perceive fine root fibres to which cling small fragments of humus. These fibres we cut off, let them lie in water for a time to soften the humus, and then examine them under slight magnification. Root hairs are absent. Instead of that, the entire surface of the fibres is covered with a tissue of fungal hyphæ. From the root fibres also radiate in all directions fine hyphæ and thick hyphal strands, to which frequently fragments of humus cling (see Fig. 25). Here we have to do with Mycorhiza,[1] and

particularly the ectotropic form of it, since the hyphæ of the fungus do not penetrate into the cells of the root, at most insinuating themselves between the epidermal cells. The Mycorhiza is made up of the root fibres, and the fungal hyphæ living in symbiosis with the root. The latter (root hairs being of course absent) absorb the water and mineral substances of which the beech (or other plants provided with Mycorhiza) has need. Probably also nitrogenous organic substances are taken up from the soil by the Mycorhiza.

We collect some humous soil from a beech wood. The material is well broken up, mixed, and sieved. We fill a few flower-pots, about 20 cm. in height, and the same in diameter, with this soil, and moderately moisten it. Some of the pots are at once employed for the following experiments (after Frank), the others being first sterilised by exposure for some hours to a temperature of 100° C. in a drying chamber. The pots are covered while in the drying chamber by pieces of glass. We now place in the soil of each pot a few beech seedlings. These are best obtained by germinating beech-nuts, just after gathering in autumn, between folds of moist blotting-paper. The seedlings are transferred to the pots after twelve to eighteen days, and these are then put in the cool-house. For watering the soil we use only distilled water.

The plants developing in sterilised soil thrive badly even in the following summer, form only root hairs (see Fig. 26), and in part die off. The rest of the plantlets grow on vigorously; in the summer all their roots are already provided with fungi. The development of Mycorhiza is thus essential for the normal development of the beech (and some other plants).

The unfavourable result of the cultures in sterilised soil is not the result of the mere sterilisation, since plants which are not dependent on symbiosis with fungi (*e.g.* oats) are easily made to flourish vigorously in sterilised soil.

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[1] See Frank, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 3 and 6.

35. Experiments with Penicillium crustaceum.

If we soak a slice of bread with water, place it in a glass dish, and leave it under a bell-glass at the ordinary room temperature, smaller forms of Mucor generally develop first of all.

The surface of the bread, however, soon takes on a greenish coloration due to a vigorous growth of Penicillium crustaceum. Under the microscope we can readily make out the mycelium and also the fertile hyphæ, branched in a penicillate manner at their ends, where they bear rows of spores (see Fig. 27).

If we wish to make culture experiments with the Penicillium, we first prepare a solution containing in every 100 c.c. of water 0·05 gr. Ammonium phosphate (prepared by saturating Phosphoric acid with ammonia, and evaporating the solution), 0·05 gr. of acid Potassium phosphate ($KH_2 PO_4$), 0·03 gr. of Magnesium sulphate and 0·01 gr. of Calcium chloride. Respecting the addition of an iron salt, see p. 58. To this solution we add the organic substances whose value as nutrient material for the Penicillium we wish to test, together with spores of the fungus. I filled, *e.g.*, four small glasses with 20 c.c. each of the solution. To *a* nothing was added; to *b* was added 0·2 gr. of grape sugar; to *c*, 0·2 gr. of Oxalic acid; to *d* 0·2 gr. of Citric acid. *a* and *b* were acidified by addition of a few drops of very dilute Sulphuric acid. In order to sow minimal quantities of spores, the best plan is to distribute some Penicillium material, grown *e.g.* on bread, in a large quantity of water, and add to the food solutions only a few c.c. of this water. The culture vessels are now covered with glazed paper, and left to themselves in darkness at the ordinary temperature.

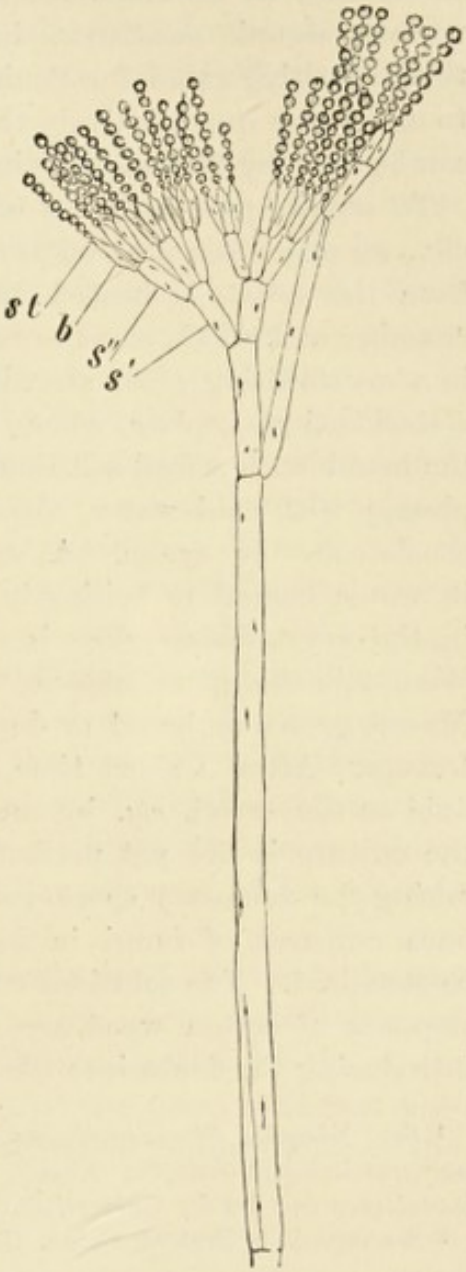

FIG. 27. — Penicillium crustaceum: Gonidiophore with tufts of branches *s' and s''*; basidia, *b*; sterigma, *st*; and gonidia. Magn. 540. (After Strasburger.)

In my experiments no Penicillium growth was observable after

more than eight days in *a* and *c*, while the fluids *b* and *d* quickly became covered with a thick coating of mould. Grape sugar and Citric acid thus serve well as food for the fungus; Oxalic acid cannot be utilised by them. In absence of organic substances, no development takes place. We can test the most various organic substances in the manner above indicated as to their nutritive value for Penicillium, and if it appears necessary to determine quantitatively the amount of fungus produced, this can be done by filtering, drying at 100° C., and weighing.[1]

To obtain pure cultures of fungi, *e.g.* of Mucor, Penicillium, etc., we may often proceed as follows:[2]— Unleavened bread, freed from the crust, is heated for one to two days in the drying chamber at 120° C., so as to become sterilised. We lay the bread in a crystallising glass sterilised by heating, and cover this with a sterilised glass plate, whose edges overhang. It is best to soak the bread with a food solution. We extract dried fruits (raisins, plums) with cold water, filter, and evaporate the solution to the consistency of syrup. A small quantity of this is dissolved in water, heated to boiling in a wash bottle, and then the bread in the crystallising glass is soaked with the boiling hot fluid.* We obtain the spore material by transferring some spores of, say, Mucor, grown on bread or dung, to boiled water by means of the forceps. After a short time, we distribute a few drops of this fluid on the bread, *e.g.* by means of a sterilised flat needle. If the culture is not yet perfectly pure, we must make a second, taking the necessary spore material from the first. In preparing pure cultures of fungi in food solutions, these must of course be sterilised. The solutions contained in small flasks closed with stoppers of cotton wood are sterilised by simple heating, or by introducing the flasks into the steam sterilising apparatus.

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[1] See Näegeli, *Sitzungsberichte d. k. bayr. Akadem. d. Wiss.*, 1879, *mathematisch-physikalische Klasse*, and Reincke, *Untersuchungen aus dem botanischen Institut der Universität Göttingen*, 1883, Heft 3.

[2] See especially Brefeld, *Botan. Untersuchungen über Schimmelpilze*, Heft 4.

36. Some Other Saprophytes.

If stems of Vicia Faba, which have been left for a long time in the fields in autumn, are soaked for several hours in spring water

* In some cases it is necessary to neutralise the acids in the fruit extract by addition of ammonia, since many fungi cannot withstand their action.

and laid on a moist filter paper in a dish which is then covered with a glass plate, a luxuriant growth of fungi will develop on them. Especially interesting is the appearance of the whitish sporophores, about 1 mm. in diameter, of Chondrioderma difforme, a Myxomycete, respecting the culture of which Strasburger [1] gives exact instructions. In experiments which I made, there also appeared on the stalks after some time the red-tinted sporophores of a Peziza.

If fresh cow-dung is placed in a dish, and covered with a bell-glass, it becomes clothed in the course of a few days with a luxuriant fungus vegetation. This is the case both when the dung is exposed to the light and when light is completely excluded. First there appear above the substratum the sporangiophores of Mucor Mucedo, which may attain the length of some centimetres, each sporangiophore bearing at its summit a roundish sporangium. It is instructive to submit this well-known fungus to microscopic examination. Later there comes to view another fungus belonging to the Mucorini, viz. Pilobolus crystallinus. This has short sporangiophores and comparatively large, hemispherical, black sporangia. After a few weeks there is found one of the cap-fungi, with a long stalk and small cap, viz. Coprinus, and finally there often appear the yellowish or brownish cup-shaped sporocarps of Ascobolus. All these fungi develop their mycelium in the dung, while the spore-producing parts project above the substratum. Since the organisms mentioned, like all fungi, contain no chlorophyll, they maintain themselves at the expense of the organic matter of the dung.

If we put a fly into water taken from a pond, it quickly decomposes, and generally representatives of the genus Saprolegnia or Achlya develop on it. Microscopical examination of the white threads surrounding the fly show us that the plant is at first unicellular. Later a club-shaped sporangium is jointed off from the end of each branch.

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[1] See Strasburger, *Practical Botany*.

37. Saccharomyces cerevisiæ.

If we tease up some pressed yeast in a drop of water on a slide, and lay on a cover glass, we shall find, on microscopic examination, that, in addition to the small, almost spherical yeast cells, large

quantities of starch are present as impurities. On addition of Iodine, the protoplasm of the yeast cells becomes brownish in colour. Dilute potash solution dissolves the contents of the yeast cells, the cell wall remaining undissolved. If we take some yeast from very actively fermenting fluid, it is seen, on microscopic examination, that the daughter cells developed by sprouting at first remain associated with their mother cells, but later become isolated.

Two glass vessels are procured of about 150 c.c. capacity. Into *a* we pour 100 c.c. of a fluid consisting of 85 parts of water, 15 parts of grape sugar, 0·2 parts of KH_2PO_4, 0·02 parts of $Ca_3(PO_4)_2$, 0·02 parts of $MgSO_4$, and 1 part of NH_4NO_3. Into *b* we pour 100 c.c. of another fluid, having the same composition as that in *a*, except that it contains no sugar. To each we now add 2 c.c. of yeast water (see Appendix), and then put them into a thermostat at a temperature of 25–30° C., shaking at frequent intervals. In *a* fairly active fermentation quickly sets in, and the fluid becomes turbid, owing to the propagation of the yeast. The solution *b* remains clear, since it contains no sugar, and consequently no source of carbon for the yeast, and the fungus is unable to propagate itself.[1] If, after allowing the fermentation to proceed for some time, we filter the fluid *a*, and collect the yeast on a previously dried and weighed filter, we can accurately determine the amount of yeast formed. If we estimate the amount of sugar in the fluid *a* before the addition of yeast, and again after the fermentation has gone on for a good time (see Section III.), we shall find that, in consequence of the fermentation, and owing of course to the vital activity of the yeast fungus, much sugar has disappeared. The sugar is expended chiefly in the production of Carbon dioxide and alcohol, and in the growth of the yeast fungus.

A solution *a* is prepared which consists of 85 parts of water, 15 parts of grape sugar, 0·2 parts of Potassium phosphate (KH_2PO_4), 0·02 parts of Calcium phosphate ($Ca_3 2PO_4$), 0·02 parts of Magnesium sulphate, and 1 part of pepsin. A solution *b* is similar in composition, except that the grape sugar is replaced by cane sugar (sugar candy). On adding a good supply of yeast, active fermentation is quickly set up in both fluids, especially if they are kept warm (25–30° C.). The yeast inverts the cane sugar, and the fermentable material thus produced rapidly undergoes decomposition into alcohol, Carbon dioxide, etc.

If fluids similar in composition to those just described are

treated with a little yeast, one solution being kept cool (at 10–15° C.), the other warm (at 25–30° C.), it will be found that in the former only slight growth and multiplication of the yeast takes place, while the second at once actively ferments, rapid multiplication of the yeast at the same time setting in.

We can easily ascertain by similar experiments that fermentation and propagation of the yeast takes place in sugar-containing solutions, in darkness as well as in light (see further Section III.).

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[1] See A. Mayer, *Lehrbuch d. Gährungschemie*, 1874, p. 107, and *Untersuchungen über die alkoholische Gährung*, 1869.

38. Bacteria.

Bacteria are very widely distributed in nature. They are transported in all directions with the dust by currents of air, and under favourable conditions display their vitality. We fill a number of Erlenmeyer's flasks with a filtered fluid containing in solution in every 100 parts of water, 1 part of grape sugar and 0·5 part of meat extract. On the walls of the flask, and especially in the meat extract, are numerous bacterium germs, and the solutions, if allowed to stand for a few days, become very turbid, owing to copious development of bacteria. By previous sterilisation, however, and arrangements for keeping the fluids sterile, this development of bacteria can be prevented.

The Erlenmeyer's flasks are first rinsed with concentrated Sulphuric acid, then with boiled distilled water. After 100–200 c.c. of the above solution have been introduced, the flasks are tightly plugged with cotton wool. Under some conditions it is better, before pouring in the solutions, to let the flasks stand for two hours in the drying chamber at 150° C. The fluid is sterilised by exposing the flasks containing it for at least two hours to a stream of steam in the Koch's steam sterilising cylinder * (to be obtained from H. Rohrbeck, Berlin).

In especially accurate work the sterilisation in the cylinder is repeated several days in succession. If in the first case spores should remain alive, they may germinate in the period preceding

* It is often sufficient merely to boil the fluid in the flasks for some time after plugging with cotton wool.

the second heating, by which those which have germinated are then killed.

When our flasks, filled with sterilised food solution, and plugged with cotton wool, are left to themselves, the fluid remains clear; even after months it still remains so, since no development of bacteria can take place.

Pure cultures of Bacterium Termo, an organism very commonly associated with putrefaction, can be comparatively easily obtained. We prepare some Cohn's normal solution. This contains in 200 parts of water 1 gr. of acid Potassium phosphate, 1 gr. of Magnesium sulphate, 2 gr. of neutral Ammonium tartrate, and 0·1 gr. of Calcium chloride. In this case simple boiling of the solution in the Erlenmeyer's flasks, closed with plugs of cotton wool, serves to ensure sterilisation. We now pour water over some peas and let them putrefy, transfer a drop of the bacterium-containing fluid to the food solution by means of a glass rod, and if Bacterium Termo develops, convey a drop of the solution to a fresh portion of the nutrient solution. We repeat this process several times, till finally a pure culture is obtained. In conveying the bacteria from one portion of nutrient solution to another, we make use of a glass rod sterilised by heating in a spirit flame. We lay it to cool on a sterilised plate of glass under a bell-glass. It is a characteristic of Bacterium Termo that the fluids in which it develops become milky in the first days, and later acquire a greenish skin. Bacterium Termo exists in the form of rodlets, which are mostly associated in pairs. These cells move to and fro by jerks. In the zooglœa, which takes the form of the above-mentioned greenish skin, are present immotile individuals.

If some malt extract (prepared by treating malt powder with water and filtering off the solution) is left to itself, the solution, originally clear, becomes turbid in the course of a few days. By microscopic examination it can be determined, in the manner to be given below, that innumerable bacteria are present in the fluid. At lower temperatures (about 15° C.) Bacterium aceti chiefly shows itself, but at higher temperatures (about 50° C.), to which we can readily expose the fluid in the thermostat, Bacterium acidi lactici is found. According to Delbrück,[1] we can obtain the Lactic acid fungus with certainty by treating 200 gr. of dry malt with 1000 gr. of water, and allowing the mixture, without filtration, to remain for some time in a thermostat at 50° C.

The hay fungus, Bacillus subtilis, is widely distributed in

nature. To obtain it with certainty, we pour a very small quantity of water over some hay, let it stand for four hours at a temperature of 36° C., pour off the fluid, without filtering, and dilute it until its specific gravity is 1·004. If the decoction is too acid, we now neutralise it with Sodium carbonate, then transfer to a flask of 800 c.c. capacity, plug this with cotton wool, and heat the fluid to boiling. It is gently boiled for an hour, and then put into the thermostat at a temperature of 36° C. In the course of one or two days a grey skin forms on the hay extract, which is composed of the zooglœa of the bacillus. The spores of the organism have survived the boiling, other bacteria which were present in the decoction have been destroyed, and we obtain in this way a pure culture

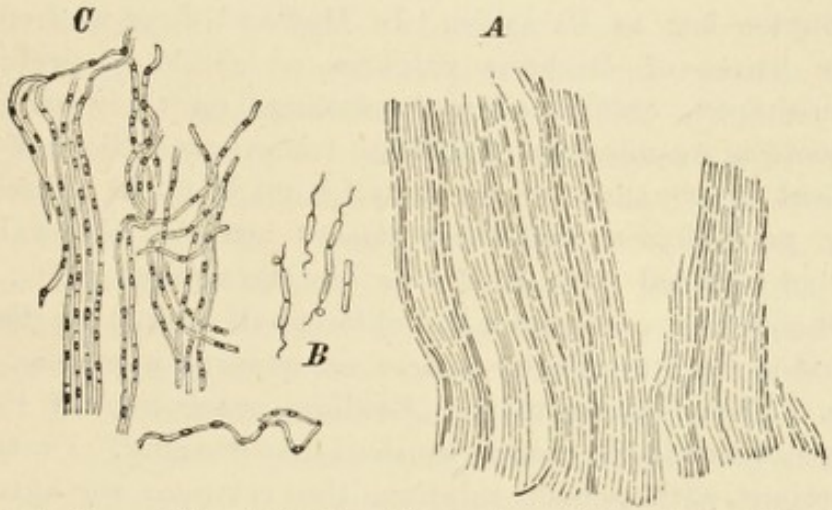

FIG. 28.—Bacillus subtilis. *A*, the skin; *B*, swarming rodlets; *C*, spore formation. *A*, magn. 500; *B*, magn. 1000; *C*, magn. 800. (After Strasburger.)

of the hay fungus. The skin is a jelly, in which are present large numbers of threads running parallel to one another, and composed of rodlets. In order to bring out very clearly the rodlets of which the threads are composed, we stain them, a plan often adopted in investigating bacteria. A small quantity of the bacterium-containing fluid is placed on a cover-glass, spread out over it and exposed to the air for some time to dry. We now rapidly draw the cover-glass several times through a spirit flame, keeping the side covered with bacteria upwards, spread out on it a drop of aqueous methyl violet or fuchsin solution (best prepared by carefully adding a small quantity of the alcoholic solution of the pigment to distilled water), and float it for twenty or thirty minutes on distilled water, preparation downwards, afterwards

allowing to dry in the air. We finally put on a drop of oil of turpentine and examine under high magnification.[2]

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[1] See Zopf, in Schenk's *Handbuch d. Botanik*, Bd. 3, p. 65.

[2] For further information respecting the investigation of Bacteria, see Flügge *Die Micro-organismen*, Leipzig, 1886, and Hueppe, *Methoden d. Bacterienforschung*, 1886.

39. Some Parasitic Fungi.

There are many fungi which are recognised as the causes of diseases in plants. They nourish themselves at the expense of the substance of living plants, and therefore must be classed not as Saprophytes but as Parasites. In May and June we frequently observe leaves of Berberis vulgaris which have protuberant cushion-shaped, orange-coloured swellings on their under side. Microscopical examination of delicate transverse sections of a Berberis leaf shows that the mesophyll is made up of palisade and spongy parenchyma, and these tissues are also observable—in somewhat modified form, it is true—in the swollen parts. Here the cell contents (protoplasm and chlorophyll grains) are disorganised, and in the intercellular spaces are present numerous fungal hyphæ. These belong to the Æcidium generation of Puccinia graminis, which is the fungus we shall first consider. On treating the sections with potash solution, the relations we have mentioned stand out with especial clearness.

On the under side of the cushions we notice peculiar cup-shaped structures, which in their development break through the tissue of the cushion, and finally even through the epidermis of the leaf. Below the cups we see a thick layer formed of hyphæ. Each cup itself consists of an investment (peridium) and the numerous spore-producing basidia, which at the base of the cup are associated to form the hymenium. On the upper side of the cushion no Æcidium cups are present, but here may be found pear-shaped structures, the spermogonia.

The spores from the Æcidium fruit of Puccinia germinate from the middle of June on various grasses (wheat, barley, oats, etc.). They attack chiefly the haulms and leaf-sheaths, and cause the familiar disease of grain known as rust. On examination of delicate transverse sections from an oat haulm covered with rust-brown stripes, due to the vegetation of the uredo-layers of Puccinia, we

shall find that numerous fungal hyphæ traverse the green tissue of the stem, disorganising the cell-contents. The mycelium further produces at particular places numerous outwardly directed branches, which perforate the epidermis of the haulm, and abstrict from their ends unicellular spores (uredospores). The uredospores then finally give rise to the teleutospore layers, which we shall not here investigate.

The common potato disease, which is so highly epidemic, is due to a fungus which belongs to the Peronosporeæ, and is known as Phytophthora infestans. It may be observed in summer on the leaves, but also in winter on the tubers, of Solanum tuberosum. If diseased potato tubers (which may be easily recognised by the presence of brownish, somewhat sunken spots on the skin) are cut up, and we leave the pieces for about two days under a bell-glass in air saturated with aqueous vapour, the cut surfaces become covered with a delicate white "mould." The mycelium of the Phytophthora is present in the diseased tubers, to begin with; it occurs in large quantities between the cells, and lives at the expense of their substance. Under the conditions described, it sends out gonidiophores to the exterior. These gonidiophores, as microscopical examination teaches, are branched in their upper parts and form sporangia, which, however, in contact with water readily fall off. If pieces of diseased potatoes are allowed to remain under the bell-glass for some time, a rich fungus vegetation develops on them, which, however, has nothing to do directly with the Phytophthora.

It is also very instructive to follow closely the course of development in a Peziza, viz. P. sclerotiorum. It is one of the Discomycetes, and causes the sclerotium disease of rape for example. I will describe my method of cultivating the fungus. A number of sclerotia are laid on the surface of moist earth contained in a flower pot. The pot is covered with a glass plate, and allowed to stand in diffused light not far from a window, care being taken that the soil does not become dry. After six to ten weeks the small, stalked sporophores of the Peziza develop from the sclerotia. A carrot is now cut up, and after the pieces have been scalded in hot water, we infect them, by means of a sterilised needle, with ripe spores from the Peziza cups, put them in a crystallising glass, and cover with a bell-glass. After a few days the spores germinate. A luxuriantly vegetating mycelium rapidly develops on the surface of the pieces of carrot, which destroys

even the inner tissue of the root not killed by the hot water. Here and there on the mycelium we observe soft white balls, which gradually increase in size, and ultimately surround themselves with a dark-coloured rind. These are sclerotia, from which new sporophores may be obtained, though not, it is true, till after a long period of dormancy. We also take a small quantity of the Peziza mycelium, vigorously vegetating on a piece of carrot, and introduce it into a small hole made in a vegetable marrow lying in a covered glass. The mycelium is able to penetrate into the living tissue; it vegetates very luxuriantly and forms sclerotia, the vegetable marrow becoming completely destroyed.[1]

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[1] Much information about parasitic fungi will be found in Frank, *Krankheiten der Pflanzen*, Breslau, 1880.

40. Lichens.

Lichens are well-known organisms which owe their existence to the association (symbiosis) of fungi and algæ. The relation in general is that the algæ in virtue of their chlorophyll produce from inorganic material, by assimilation, the organic material necessary both for their own existence and that of the fungi, while the fungi afford the algæ protection, more especially against desiccation. The lichen thallus may be homoiomerous or heteromerous in construction. To acquaint ourselves with the structure of these remarkable organisms, we will take for examination a representative of the latter group of lichens, viz. Usnea barbata.

We may use either fresh material, or, as I have found suitable, herbarium material softened in water. On submitting to microscopic examination a delicate transverse section of a strong branch of the thallus, we at once see that it is differentiated into pith and cortex. The elements of both layers are much branched fungal hyphæ. The cortex, and also an axile strand of the pith, are dense in structure, while the peripheral region of the pith is composed of hyphæ loosely arranged, and with air-containing spaces between them. The green algæ are easily seen at the boundary between pith and cortex. They form there a special zone, which is everywhere traversed by hyphæ running from pith to cortex. If we desire to investigate the structure of other lichens, it will be of

special interest to examine the sporophores of varieties of Cladonia and the dorsiventral thallus of species of Sticta.[1]

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[1] On the structure and mode of life of lichens, see de Bary, *Comparative Morphology and Biology of the Fungi.*

41. Experiments with Carnivorous Plants.

Drosera plants which it is desired to employ for physiological experiments are best cultivated in flat earthen vessels on moist Sphagnum under bell-glasses. The Droseras are frequently to be found in large quantities on swampy, boggy soil ; for cultures we need only lay a clump of the plants on the Sphagnum. It is well known that when the leaf of Drosera is stimulated, the lamina curves inwards and the tentacles lay themselves together. We first place a fragment of raw beef of the size of a pin's head on the middle of the leaf of a healthy specimen of Drosera rotundifolia. After some time (in my experiments at 20° C. in twenty-four hours) all, or almost all, the tentacles have bent inwards. They now surround the fragments of beef ; the secretion of their glandular heads exerts a solvent action on the proteid ;[1] but ultimately (in my experiments at the end of forty-eight hours) the tentacles straighten out again. If we place on a Drosera leaf non-nitrogenous inorganic or organic bodies (I experimented with fragments of glass and pellets of paper), the tentacles certainly bend inwards, as before, but it is readily determined that such substances induce movements much more slowly than fragments of meat. It is particularly to be observed that whether chemical stimulus (by fragments of meat) or contact stimulus (by fragments of glass) is applied to the Drosera leaf, tentacles are thrown into movement, owing to transmission of stimuli, whose glandular tips have not been directly brought into contact with the substance acting as an irritant.

If we examine tentacles of Drosera under the microscope (we may mount them in a drop of chloral hydrate solution to render the tissues transparent), we find that the stalk of the tentacle is composed of longitudinally elongated cells ; the stronger stalks are traversed by an axile strand of spiral vessels. The head of the tentacle consists centrally of spirally thickened elements, which

are surrounded by radially elongated cells arranged in a fan-like manner (see Fig. 30).

It is interesting to observe the phenomena of *aggregation*, which under certain conditions are very strikingly exhibited in the cells of the tentacles of Drosera.[2] We take a tentacle from a Drosera which has always been exposed to intense illumination, so as to

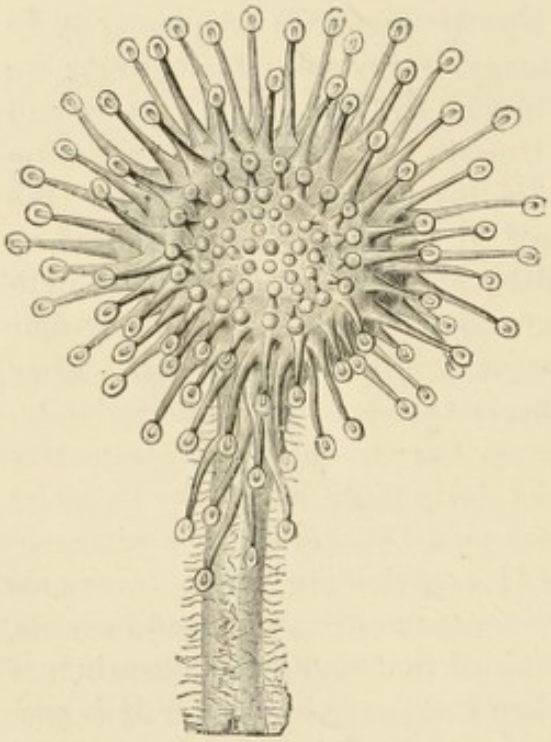

FIG. 29.—Leaf of Drosera rotundifolia, seen from above. Magnified 4 times. (After Darwin.)

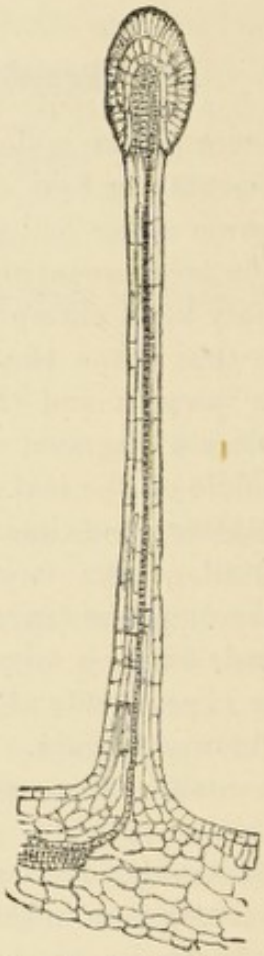

FIG. 30.—Tentacle, with grandular head, of Drosera rotundifolia. Magn. 60. (After Strasburger.)

favour the development of red pigments in the tentacle cells, mount it in a drop of water, and examine microscopically. We focus a cell of the outer layer of the tissue, and observe parietal protoplasm and red sap uniformly filling the cells. We now bring in contact with the secreting glandular heads of a few of the tentacles of our plant very small pieces of boiled proteid. After twelve to twenty hours we cut off the stimulated tentacles and examine them. We see the parietal protoplasm. This now

encloses colourless cell-sap, in which, however, the variously shaped aggregations, in the form of intensely red-coloured masses, are easy to make out. These aggregations have a wall, the nature of which has not yet been accurately determined; at least, I do not consider the statements of de Vries on the subject fully conclusive. Within them cell-sap is present. In the production of these structures the cell-sap, originally uniformly distributed in the cell, must have separated into two parts, one very rich in pigment, the other clear like water. How this comes about is not yet adequately determined. When Ammonium carbonate is brought into contact with tentacles of Drosera, processes of aggregation are also exhibited, in which, however, precipitations of proteid have been observed (H. de Vries).

Plants of Dionæa muscipula to be employed for physiological experiments are most conveniently cultivated on pieces of wet peat under a bell-glass. I shall not here consider the morphology of the leaf of Dionæa, but simply describe carefully a few easily conducted experiments. If the filaments present on the upper side of the Dionæa leaf are touched, say with a small splinter of wood, the leaf at once closes up. Soon, however (in my experiments at the end of twenty-four hours), the wings of the leaf will be found to have expanded again. If fragments of raw meat are placed on a Dionæa leaf so as to touch the filaments, there is an immediate closing movement. In this case, however, the leaf remains closed for a long time (in experiments conducted by me for more than eight days), thus differing from leaves induced to close by placing non-nitrogenous bodies on their surface (fragments of glass, paper pellets), or by simply touching their filaments. When a Dionæa leaf, on which have been placed fragments of meat, opens again, it is seen that the meat is more or less disorganised and dissolved, this result being due to a secretion produced by glands present on the upper side of the leaf. This

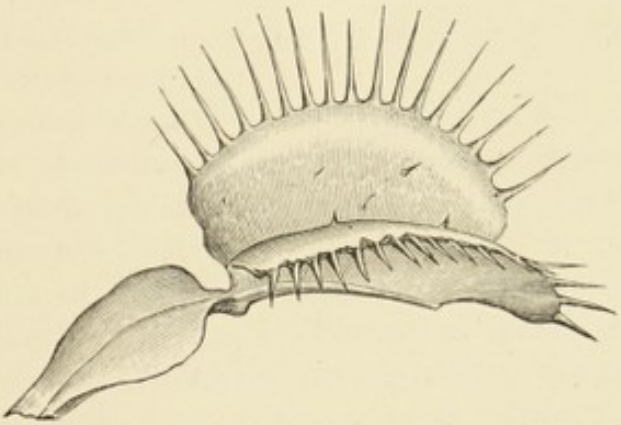

FIG. 31.—Leaf of Dionæa muscipula, expanded and seen from the side. (After Darwin.)

secretion has an acid reaction. It is often still found in moderate quantities on the surface of leaves which have been fed with meat, and in course of time have opened again. Non-nitrogenous bodies do not induce the production of a glandular secretion when placed on a Dionæa leaf; the upper surface of the leaf wings, in the absence of proteid substances, remains dry.[3]

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[1] A peptonising ferment has been isolated from Drosera leaves by Reess and Will. See *Botan. Zeitung*, 1876, Nr. 44.

[2] Literature. Darwin, *Insectivorous Plants*, 1876; Schimper, *Botan. Zeitung*, 1882; H. de Vries, *Botan. Zeitung*, 1886; Klemm, *Flora*, 1892.

[3] For a detailed account of the behaviour of these plants, see Darwin, *Insectivorous Plants*. For a general view of the subject, consult Detmer, *Lehrbuch der Pflanzenphysiologie*, 1883, pp. 65 and 282.

SECOND SECTION.

The Molecular Forces in Plants.

I. THE MOST IMPORTANT ORGANISED STRUCTURES OF PLANT CELLS.

42. The Membranes of Plant Cells.

THE membranes of plant cells are by no means always composed chiefly of cellulose, but are frequently more or less rich in other substances, which we may call in general *imbedded substances* (*Einlagerungskörper*). To such deposition of foreign substances is due, *e.g.*, the cuticularisation and lignification of membranes, with which we shall deal further in the sequel. In many other cases, however, cellulose does form the most essential constituent of the cell membranes, as is immediately shown by their behaviour towards various reagents.

We place on a slide in a drop of water hairs from the seed of a Gossypium or a few fibres of cotton wool. It is readily determined that the hairs, which in general are conical in form, have comparatively thick membranes, and only stain brownish on an addition of iodised solution of Potassium iodide (prepared by dissolving 0·05 gr. of Iodine and 0·2 gr. of Potassium iodide in 15 gr. of water). We now run in Sulphuric acid from the edge of the cover-glass (a mixture of 2 parts of concentrated Sulphuric acid and 1 part of water), and at once observe that the hairs stain blue. The membranes of other cells which, like those of cotton hairs, consist chiefly of cellulose, give the same reaction. Similarly all cell membranes which consist essentially of cellulose, stain violet on treatment with iodised solution of Zinc chloride, as we may easily ascertain by treating a few fibres of cotton wool on the slide with the reagent. *Chlor-zinc-iod.* solution is prepared as follows: We dissolve pure rod zinc in pure Hydrochloric acid, at the ordinary temperature, until saturated, evaporate in presence of excess of metallic zinc to the consistency of Sulphuric acid, add

as much Potassium iodide as can be absorbed, and finally saturate the solution with Iodine.

We can easily convince ourselves of the existence of the cuticle by study of a transverse section of a one-year-old stem of Viscum album, or of a leaf of an Aloe. The cuticle is also very strongly developed on the surface of the leaf of Ilex aquifolium. A very delicate transverse section through the midrib of an Ilex leaf teaches us that the epidermal cells of the under side of the leaf have a half-moon shaped lumen. The cuticularised layers of the cell membranes extend into the side walls of the cells, and are covered on the outside by the cuticle proper.[1] The cuticle of most leaves and of other parts of plants is thin and very delicate in constitution.

To familiarise ourselves with cork tissue, we may examine delicate transverse sections of the skin of a potato, of an ordinary bottle cork, or of one of the older stems (say, 1 cm. thick) of a plant of Aristolochia Sipho. The cork cells, arranged in radial rows, are more or less tabular in form. On studying the periderm of Aristolochia, it is seen that broader zones of wide cork cells, and thinner zones of narrow cork cells, alternate with one another. Concentrated potash solution stains both cuticularised and corky membranes yellowish. This coloration is intensified by warming the preparations.[2]

We prepare a transverse section of a twig of Tilia a few mm. in thickness. The microscopical structure is easy to understand. We are here chiefly concerned with the wood and the bast of the vascular bundles, the former being composed principally of vessels of varying diameter and wood fibres. The bast masses taper in a wedge-like manner, the apices of the wedges being directed towards the cortex, while the wedge-shaped ends of the primary medullary rays, which alternate with the masses of bast, turn their apices towards the wood. In the bast masses bright strips, composed of very much thickened bast fibres, alternate with dark strips of soft bast. We now lay our section on the slide, in a drop of alcoholic phloroglucin solution. After a little time, when the alcohol has evaporated, we moisten the section with concentrated Hydrochloric acid, and examine it under the microscope. All the lignified elements have stained red, while the unlignified elements remain unstained, so that phloroglucin forms an excellent reagent for woody material.[3] It is particularly to be observed that not only the elements of the wood proper of the

fibro-vascular bundles are coloured red, but also the elements of the bast fibre bands between the strips of soft bast, and therefore these also have lignified membranes.

If we treat sections of twigs of Fagus sylvatica, 2 mm. thick, with phloroglucin and Hydrochloric acid as above described, it will be found that lignification has taken place in all the following tissues: in the whole of the pith, in the medullary rays, in the wood of the vascular bundles, and in the masses of bast fibres which adjoin the soft bast on the outside. The elements of the cambium, of the soft bast, of the cortex, and of the periderm, do not stain red, and therefore are not lignified. Another very useful reagent for woody material is aniline sulphate. We prepare a concentrated aqueous solution of this substance, add to it some Sulphuric acid, and treat the section on the slide with a drop of the reagent. The lignified elements quickly become more or less yellow in colour. I found, *e.g.*, that the bast fibres in twigs of Fagus sylvatica become bright golden-yellow in colour when treated with aniline sulphate. On treatment with an aqueous solution of methyl green, lignified elements take on a beautiful greenish-blue colour, while the unlignified elements for the most part stain blue.

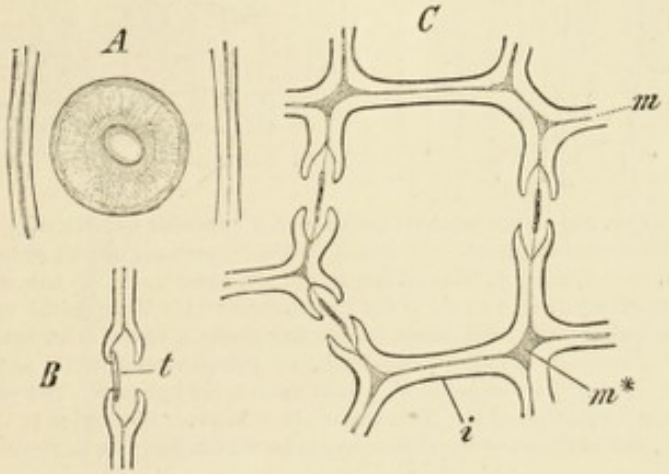

FIG. 32.—Pinus sylvestris. *A*, a bordered pit of a tracheide in surface view; *B*, a bordered pit in longitudinal tangential section, *t*, the torus; *C*, the transverse section of an entire tracheide; *m*, middle lamella, *i*, the limiting membrane. Magn. 540. (After Strasburger.)

To familiarise ourselves with the most important forms of thickening in the elements of woody tissue, the following observations must be made. The bordered pits of the tracheides of coniferous wood are best studied in delicate transverse sections and radial longitudinal sections from the peripheral parts of the wood of old stems of Pinus sylvestris which have been preserved in alcohol (see Fig. 32). The tracheides are elongated, and their tapering ends interlock with each other. Their bordered pits are easily recognised on the radial walls, the walls, viz., which are turned towards the medullary rays. If we prepare delicate radial

longitudinal sections through the secondary wood of a twig of Aristolochia Sipho about 1 cm. in thickness, we shall observe numerous tracheides with bordered pits, together with narrow and very wide vessels with bordered pits and annular diaphragms. If we examine radial longitudinal sections of twigs of Berberis vulgaris, we shall observe that the entire wood consists almost exclusively of vessels with bordered pits, and of wood fibres. The

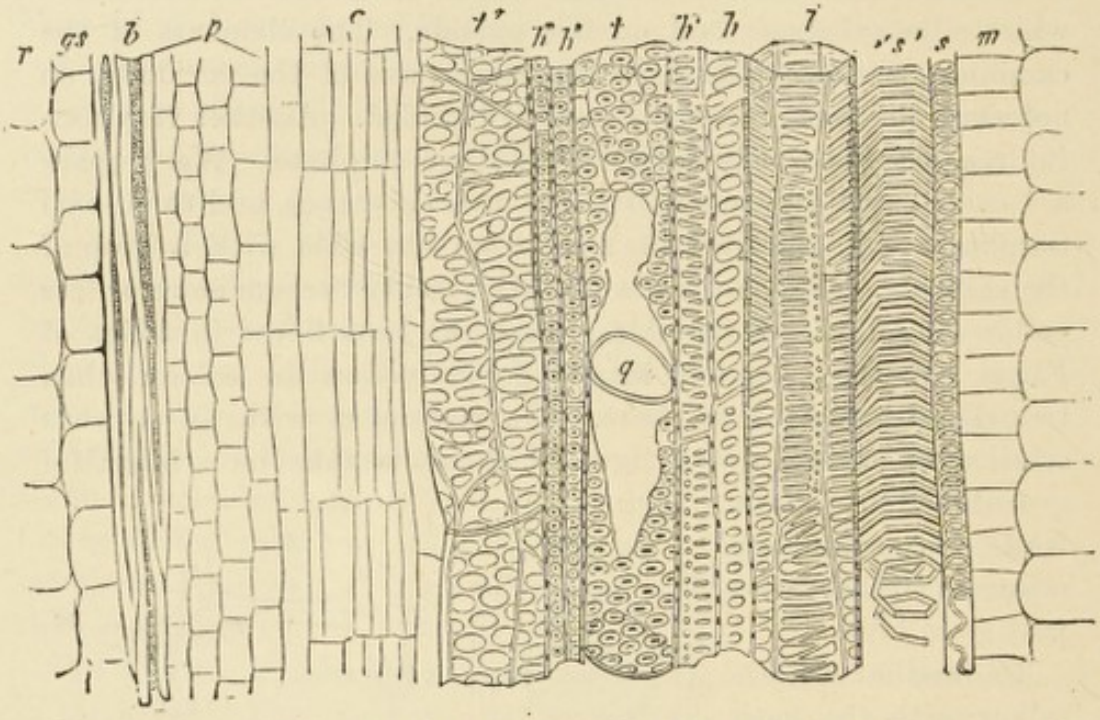

FIG. 33.—Longitudinal section of a vascular bundle of the fully elongated hypocotyl of, Ricinus communis. *r*, cortical parenchyma; *gs*, bundle sheath; *m*, medullary parenchyma; *b*, bast fibres; *p*, bast parenchyma; *c*, cambium. In the wood the elements develop successively from *s* to *t'*; *s*, the first narrow very long spiral vessel, *s'*, wide spiral vessel, the spiral band in both cases partly unrolled; *l*, vessel with scalariform and, in part, reticulate thickening; *h* and *h'*, wood cells; *t* pitted vessel, with, at *q*, an absorbed transverse wall; *h''* and *h'''*, wood cells; *t'*, pitted vessel, still young. The pits first show the outer border, the formation of the pore takes place later. We notice in the wall of the vessel in *l*, *t*, and *t'*, the outlines of neighbouring cells which have been removed. (After Sachs.)

true spiral vessels of the primary wood of the vascular bundles may be very beautifully seen, in addition to other elements (pitted vessels, wood fibres, etc.), if we examine radial longitudinal sections of the stem of Helianthus annuus or of the fully elongated hypocotyl of Ricinus communis. We employ alcohol material, or in the case of Helianthus dried material will serve[4] (see Fig. 33).

It is instructive to separate the elements of the wood from one another by the maceration method. We place a few crystals of

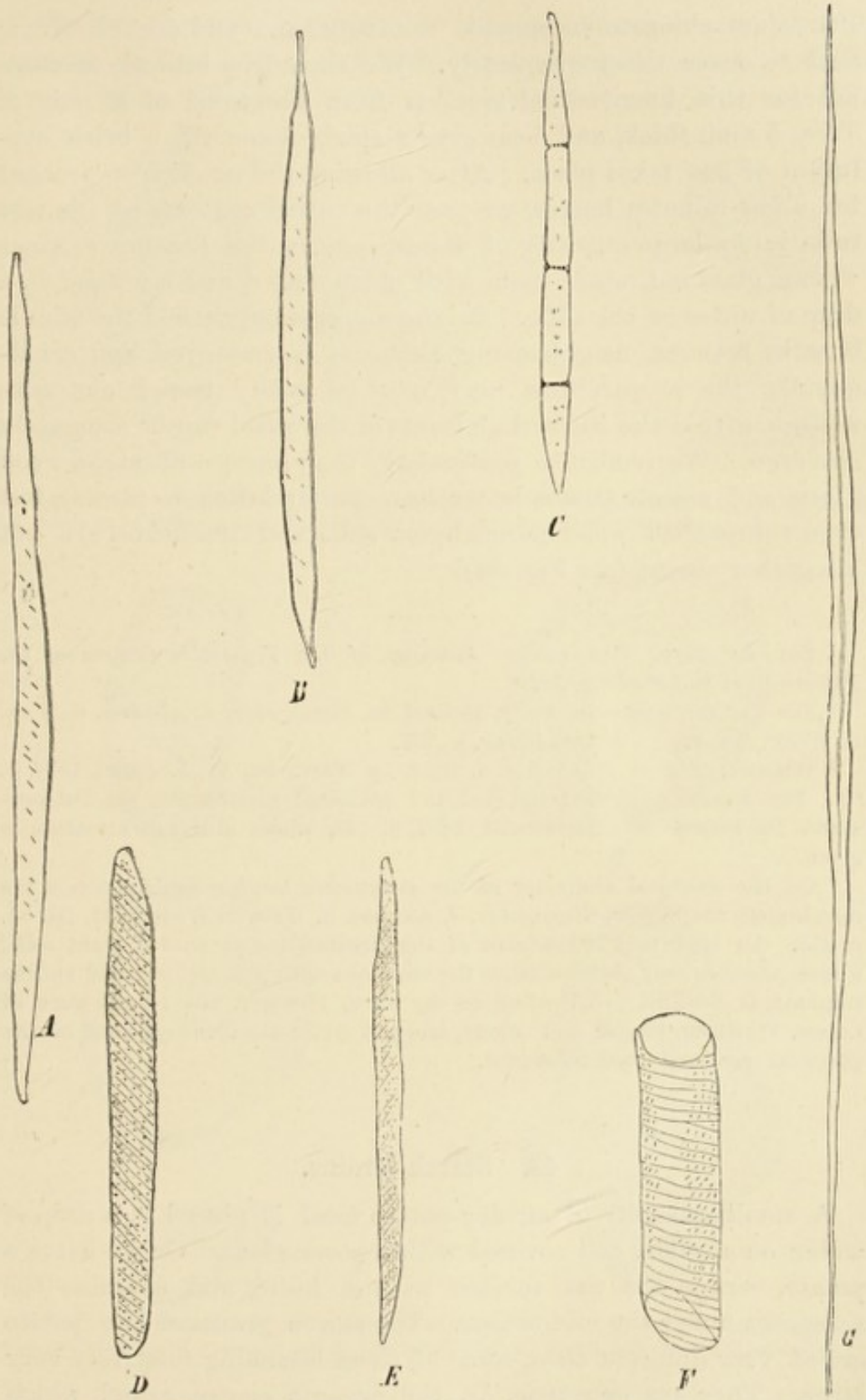

FIG. 34.—Tilia parvifolia. Elements of the secondary wood and bast isolated by maceration. *A* and *B*, wood fibres (libriform fibres); *C*, wood parenchyma; *D* and *E*, tracheides; *F*, segment of a vessel; *G*, bast fibre. Magn. 180. (After Strasburger.)

Potassium chlorate in a wide test tube, and add enough Nitric acid to cover them completely. We then drop into the mixture not too thin longitudinal sections from the wood of a twig of Tilia, 5 mm. thick, and heat over a spirit flame till a brisk evolution of gas takes place. After allowing the reaction to proceed for a few minutes longer, we pour the entire contents of the test tube into a large quantity of water, remove the floating sections with a glass rod, wash them with clean water, and lay them in a drop of water on the slide. By the maceration method the middle lamella between neighbouring elements is destroyed, and consequently the preparations may now be easily teased out with needles, so that the isolated elements of the wood can be separately observed. We recognise particularly the presence of many wood fibres and vessels (these latter have partly fallen to pieces), but even thin-walled wood parenchyma cells and tracheides are not altogether absent (see Fig. 34).

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[1] See de Bary, *Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns*, p. 74.

[2] For further reactions, see v. Höhnel in *Sitzungsber. d. Akadem. d. Wiss. zu Wien.*, Bd. 76, Erste Abtheilung, p. 507.

[3] Wiesner, *Sitzber. d. Akadem. d. Wiss. zu Wien.*, Bd. 77, 1. Abth., 1878, p. 60. For details respecting lignified and suberised membranes, see Zimmermann, *Die botanische Mikrotechnik*, 1892, p. 140, where also the literature is given.

[4] On the chemical character of the substances causing lignification of the membranes, see Singer, *Sitzungsber. d. Akadem. d. Wiss. zu Wien*, 1882, Bd. 85, p. 345. An important constituent of the lignified membranes of plant cells, whose presence may perhaps cause the reactions with phloroglucin and aniline sulphate, is vanillin. According to my view, however, the recent work of Lange, Thomsen, Hegler and others, has not yet settled the question of the chemical nature of wood substance.

43. Starch Grains.

A small quantity of air-dry potato meal is placed in a drop of water on a slide, and covered with a cover-glass. Or we halve a potato, scrape the cut surface with a knife, and examine the scrapings under the microscope. The starch grains of the potato are of very different sizes, some of them becoming relatively very large. They are excentric, *i.e.* the organic centre round which the layers are arranged does not correspond with the geometrical centre (see Fig. 35).

We cut through a rhizome of Canna indica, scrape the cut surface with a knife, and then place a small quantity of the scrapings in a drop of water on a slide and cover with a cover-

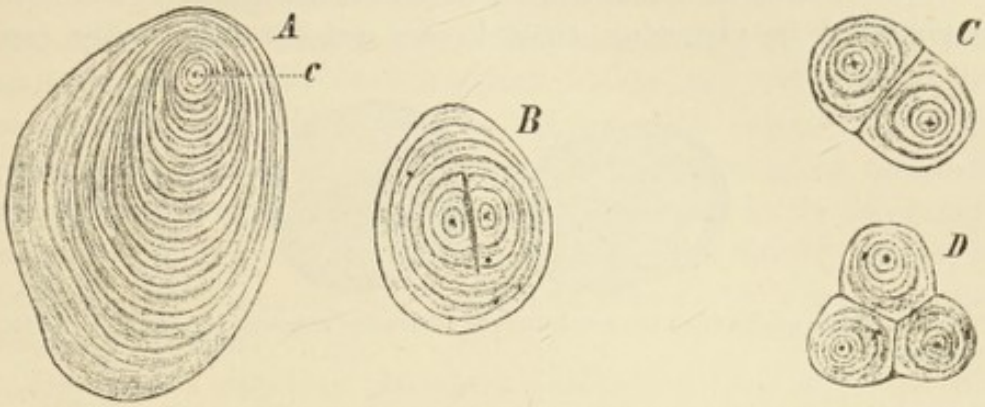

FIG. 35.—Starch grains from a potato. *A*, simple grain; *B*, semi-compound grain; *C* and *D*, compound grains; *c*, the organic nucleus. Magn. 540. (After Strasburger.)

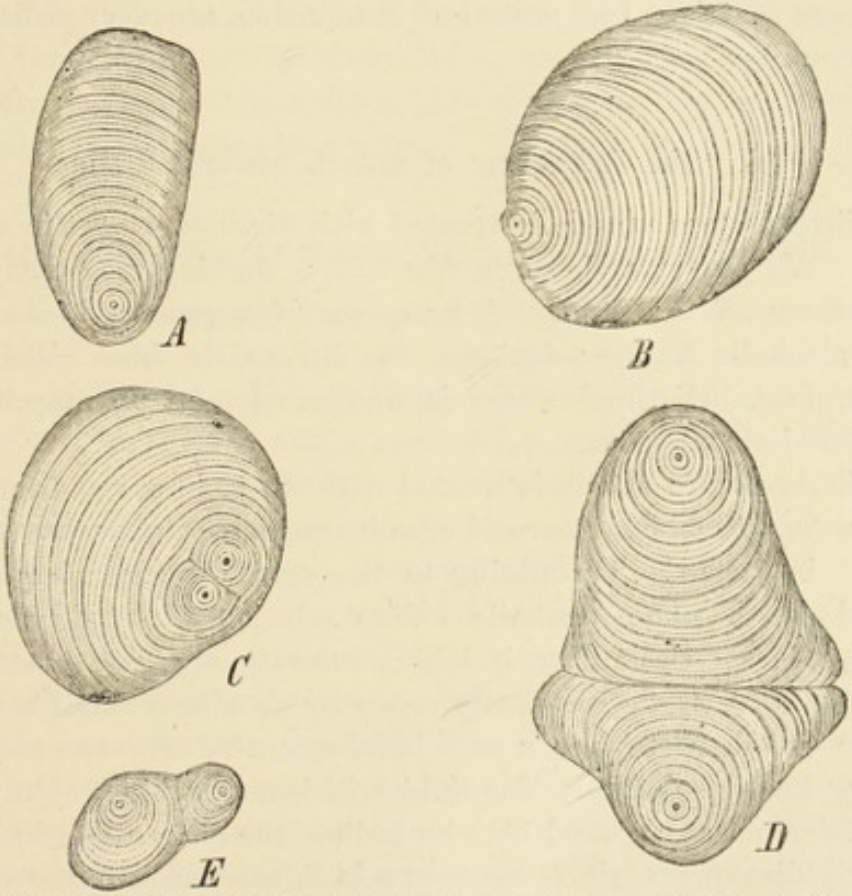

FIG. 36.—Starch grains from the rhizome of Canna indica. *A* and *B*, simple grains; *C*, a semi-compound grain; *D* and *E*, compound grains. Magn. 540. (After Strasburger.)

glass. On microscopic examination we perceive large numbers of beautifully layered starch grains, very excentric in construction, and of considerable size (see Fig. 36).

The starch grains from the cotyledons of seeds of Phaseolus vulgaris are centric. If we examine them in a drop of water, we observe in the middle of each grain a cavity, which, however, is due to the action of the water, for if the starch grains of the bean are observed in glycerine, they do not exhibit this cavity (see

FIG. 37.—Starch grains from the cotyledons of Phaseolus vulgaris. Magn. 540. (After Strasburger.)

Fig. 37). The starch grains from the endosperm of Triticum present themselves as round structures of very different sizes. They are centric. Indications of lamination are very difficult to make out.

44. The Behaviour of Starch towards Iodine.

A little potato starch is treated with distilled water in a test-tube. We put it aside for a few hours, shaking frequently, and then filter off the fluid. If, by means of a glass rod, we add a little alcoholic Iodine solution to the filtrate, the blue colour does not appear. Distilled water is unable to take up starch from uninjured starch grains.

A little potato starch is treated with water in a test-tube. On warming, a turbid mixture of starch substance and water (starch paste) is formed. On adding to the starch paste, after it has cooled down, some alcoholic Iodine solution, the fluid assumes a very characteristic beautiful blue coloration. This reaction is very delicate, as we can easily prove by pouring a small quantity of the starch paste into a very large quantity of water, and then adding Iodine solution. The fluid still becomes blue. On warming starch paste coloured blue by Iodine, the characteristic colour quickly disappears, since water at a high temperature can dissolve a fairly large quantity of Iodine, and hence, under the conditions described, can withdraw it from the starch substance. When the starch paste cools, the blue colour reappears.

A very small quantity of potato starch, or a small quantity of some other kind of starch, is mounted in a drop of water on a

slide. We now place at the edge of the cover-glass a drop of some Iodine reagent (Iodine water, iodised Potassium iodide solution, or dilute iodised alcohol). To prepare Iodine water we treat Iodine with distilled water, and put aside for a few days. To prepare the iodised Potassium iodide solution we add 60 parts of water to 3 parts of Potassium iodide, and then add 1 part of Iodine. The solution may be diluted with water. The Iodine reagent, being placed at the edge of the cover-glass, gradually advances towards the starch grains, and it may easily be observed under the microscope that these at first stain faintly bluish, but little by little become more intensely blue as they take up more and more Iodine.

Dry potato starch is placed on a slide in a drop of Iodine tincture, freshly prepared by dissolving Iodine in absolute alcohol. On microscopic examination it is seen that the starch grains do not become blue, but brownish in colour. If we allow access of water, the characteristic blue tint appears. The starch grains, therefore, are only able to stain blue with Iodine when they have imbibed a fair quantity of water.

45. Behaviour of Starch Grains in Polarised Light.

It is very instructive to investigate the phenomena studied by von Mohl[1] and Nägeli,[2] which are exhibited by starch grains when examined in polarised light. We require for this purpose a polarising apparatus and a microscope stand with a sufficiently high stage. The polariser is accommodated below the stage. For the analyser it is best to use the ocular analyser of Abbe, which, as also the polariser, may be obtained of Zeiss, Jena.

The starch grains are mounted on the slide in the usual manner, in a drop of water, and covered with a cover-glass. When the planes of polarisation of the polariser and analyser are parallel to one another, the field of view is bright, and they must be so arranged for focussing the preparation. When the Nicols are crossed (by rotating the ocular analyser), the field of view becomes dark. The amylum grains stand out very brightly from the dark background, and bear a black cross. Very beautiful colour effects are to be observed, if we examine starch grains through a polarising microscope after plates of gypsum of a particular description have been interposed between the object and the polariser.[3]

I have observed the behaviour in polarised light of a whole series of different kinds of starch grains; potato starch gave the most beautiful figures of all. We can no longer retain the view that the micellæ of starch grains, and similar organised plant structures, are of the nature of optically biaxial crystals. The characteristic behaviour of organised plant structures in polarised light must be otherwise explained.[4] I do not, however, as I have already emphasised elsewhere, reject the theory that organised plant structures consist of micellæ.[5]

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[1] See H. v. Mohl, *Botan. Zeitung*, 1858, p. 1.

[2] See Nägeli, *Sitzungsberichte d. Akadem. d. Wiss. zu München*, 1862, Bd. 1, p. 311.

[3] See detailed account in Nägeli and Schwendener's *Microscope*.

[4] See Strasburger, *Bau und Wachsthum d. Zellhäute*, 1882, p. 208; and Zimmermann, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 2, p. xvii.

[5] See Detmer, *Lehrbuch der Pflanzenphysiologie*, 1883, p. 71.

46. The Protoplasmic Structures of Plant Cells.

All the albuminous and organised constituents of the cell contents are to be regarded as protoplasmic structures (the living protoplasm proper, the nuclei, leucoplasts, and the protein grains, etc.). The chlorophyll bodies have already been discussed in 5, and we shall return later to other protoplasmic structures impregnated with pigments.

The appearance which the protoplasm (cytoplasm) of the cells presents under the microscope is essentially dependent on the number and size of the vacuoles filled with cell-sap which are present in it. The presence of many small vacuoles in the plasma gives it a foamy appearance; while the protoplasm of many cells, especially of mature ones, exhibits a single continuous sap cavity, such as is represented in Fig. 5, p. 18, and in Fig. 38. We mount in a drop of water, without special preparation, young leaves from the end bud of an Elodea, and examine under the microscope. The parietal layer of protoplasm, lining the inside of the cell membrane of each cell, is easily made out, as also the mass of protoplasm collected round the nucleus. These two are connected by threads of protoplasm which traverse the vacuole of the cell. In the protoplasm numerous chlorophyll bodies are readily seen. The staminal hairs of Tradescantia are made up of single rows of cells containing a violet cell-sap. These cells are similar in their

protoplasmic construction to those of Elodea (see Fig. 38), as may readily be determined if we remove with the forceps a tuft of hairs from opening flowers of Tradescantia virginica, or a related species, and examine them under the microscope.

The now well-established fact that the protoplasmic masses of neighbouring cells are almost universally connected with each other by delicate plasmic threads traversing the cell-walls,[1] is of the utmost importance.

FIG. 38.—A cell of a stamina, hair of Tradescantia virginical Magn. 240. (After Strasburger.)

We employ for investigation stems of Rhamnus Frangula, at least 1 cm. in thickness. These, after Strasburger, are treated as follows: We remove the periderm, and prepare very delicate tangential longitudinal sections of the green cortex. In examining the structure of the secondary cortex, we direct our attention particularly to the chlorophyll-containing bast parenchyma, whose walls are provided with unbordered pits. The elements of this bast parenchyma are rectangular in form. In addition we see the elongated bast fibres, and the spindle-shaped sections of the medullary rays. We now bring fresh sections from the secondary cortex on to a cover-glass, add a drop of concentrated Sulphuric acid, and after a few seconds dip the cover-glass into a vessel of fresh water, so as to wash the sections rapidly and as completely as possible. We then stain the sections with aqueous solution of aniline blue, wash with water, and mount in glycerine diluted with water. Instead of aniline blue, we may employ with advantage picric-aniline blue, prepared by dissolving Picric acid to saturation in 5 per cent. alcohol, and adding aniline blue till the fluid is bluish-green in colour. In successful preparations the walls of the bast parenchyma cells are so much swollen that they have about the same diameter as the contracted and stained plasmic cell contents, and the middle lamellæ are also much swollen. Not all preparations, and not all the cells of a preparation, are suitable for examination. The cells must be completely intact, and the fixation by means of Sulphuric acid must have been effected sufficiently quickly.

The protoplasm of the individual cells of the parenchyma appears smooth in outline at places where it bordered on a cell-wall provided with very fine pores; it shows thicker or thinner processes where the adjacent cell-wall possessed wider pits. The protoplasmic processes of neighbouring cells correspond with one another. If we consider the swollen closing membrane separating two specially broad plasmic processes, directed towards each other, we find running between them a number of extremely fine granular threads. These are the plasmic filaments, which connect the living protoplasm of the two cells. Where the adjoining protoplasmic surfaces appear smooth, the middle layers of the cell-wall between them are seen to be traversed, often throughout their entire extent, by filaments, which at their middle appear somewhat swollen.

The protoplasm is always bounded on its surface, even when surrounded by a cell-wall, by a hyaline layer, the ectoplasm (*Hautschicht*) or hyaloplasm. This layer is, however, also present at places where the protoplasm borders on the cell-sap. The bulk of the protoplasm is formed by the granular layer, which is distinguished by its great richness in water and by its power of movement, and further by the fact that it contains microsomata, and often also chlorophyll bodies, etc. The hyaloplasm of most cells is too delicate to be observed directly. In some cases, however, its presence may easily be determined, and it is specially well developed in the internodal cells of Nitella, a genus of Algæ of which representatives may frequently be met with, vigorously developed, in waters poor in lime. The masses of the granular layer of the protoplasm are here in active rotation, whilst the hyaloplasm does not share this movement.

I have often and emphatically pointed out[2] that there is no kind of identity between dead and living proteid molecules of the protoplasm. Certain observations of Loew and Bokorny,[3] which we must consider in some detail, are in harmony with this view. We prepare a solution of potash of 1·333 sp. gr., and add 13 c.c. of it to 10 c.c. of ammonia of 0·960 sp. gr. The mixture is made up to 100 c.c. We further prepare a 1 per cent. solution of Silver nitrate. For use, 1 c.c. each of the potash-ammonia fluid and the Silver solution are mixed, and diluted up to 1 litre. We now place in a litre of the alkaline Silver solution a few filaments of Spirogyra (other cells show the reaction in question in the same manner, but not so clearly), and allow them to remain in it for some time (at

a higher temperature, *e.g.* 30° C., for about three hours, at a lower for a longer time), and then examine microscopically. The protoplasm of the cells, owing to reduction of the Silver salt in the solution, has stained black ; but it is specially important to observe that this reaction only takes place when the cells were living at the commencement of the experiment. Spirogyra cells killed by heat, or alcohol, or otherwise, only acquire a yellow or brown colour on exposure to the alkaline Silver solution. The protoplasm of originally living cells of Spirogyra blackens somewhat more slowly, but even better, if we lay a few filaments of the alga in a solution composed of 10 mg. of Silver nitrate and 5 c.c. of lime water, to 1 litre of water. In this case, access of air containing Carbon dioxide is to be carefully avoided during the reaction.

According to my view, the blackening of the protoplasm of cells brought into contact, while living, with the Silver solutions, is at least frequently due essentially to the reduction of the Silver salt by non-nitrogenous bodies, of the nature of aldehydes, which, together with amido-acids and acid amides, are produced in the decomposition of the living proteid molecules. Dead proteid molecules have no such effect on the Silver solution, since they do not decompose like the living proteid molecules.

We now pass on to the cell-nucleus. That this contains proteid substances is shown by its behaviour towards reagents. In contact with iodised Potassium iodide solution (prepared by dissolving 0·050 grm. of Iodine and 0·200 grm. of Potassium iodide in 15 c.c. of distilled water), the cell-nucleus takes on a yellowish colour. Methyl green Acetic acid (prepared by adding methyl green to 1 per cent. Acetic acid) stains the nucleus very beautifully. On treating the cells with these reagents the nuclei stand out more conspicuously, which is often a matter of considerable importance. The nuclei of the epidermal cells of Aspidistra leaves stain very beautifully with methyl green Acetic acid, as I have often found. To familiarise ourselves with the reaction of nuclei towards iodised Potassium iodide solution, we investigate the epidermal cells of the leaf of Escheveria globosa, or delicate transverse sections through the first leaf sheath of young maize seedlings. In the cells of the parenchyma of the sheath are present fairly large cell-nuclei. Beautifully developed nuclei are present in the cells adjoining the stomata on the under side of the leaf of Tradescantia virginica. We can easily ascertain that this is the case by microscopic examination of strips of epidermis from the leaves.

The starch-forming corpuscles, leucoplasts, have a special interest for us. Their function, as Schimper first demonstrated,[4] is the regeneration of starch grains from dissolved carbohydrates which are in migration, or have already passed over into receptacles for reserve material. The largest and most beautiful starch-forming corpuscles with which we are acquainted occur in the pseudo-tubers of Phajus grandifolius, one of the Orchidaceæ. For examination we select a not too old tuber, halve it, and prepare from the crown thin longitudinal sections including the green surface of the tuber. I have satisfied myself that it is best to transfer the sections rapidly to concentrated Picric acid, and examine them in this. The starch-forming corpuscles of the cells of the inner parts of the sections are colourless; towards the outside the starch-formers certainly become larger, but their protoplasmic matrix is impregnated with chlorophyll. The leucoplasts, when seen in profile (see Fig. 39), appear rod-shaped. They have assumed a yellowish colour owing to the treatment with the Picric acid, while the larger or smaller starch grains seated on them remain unstained.

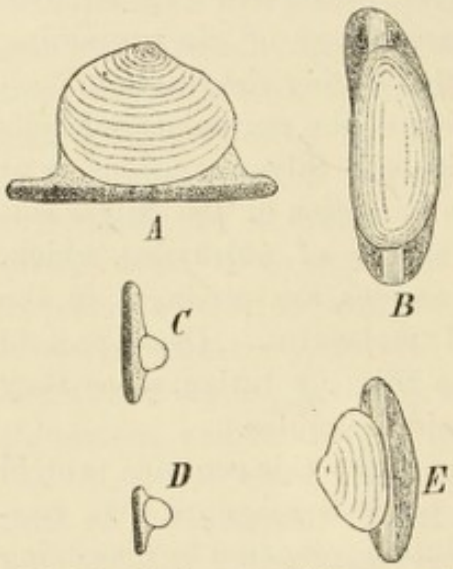

Fig. 39.—Phajus grandifolius, starch formers from the tubes. *A*, *C*, *D* and *E*, seen from the side; *B*, from above. Magn. 540. (After Strasburger.)

We now proceed to investigate the protoplasmic structures of resting organs. Above all, we are interested in the forms in which reserve proteid materials occur in seeds. We first of all take for examination a lupin seed, halve it, moisten the cut surface of the cotyledons, and examine delicate sections in water. We find in the cells numerous small aleurone or protein grains, lying closely packed together, which have become somewhat changed in form under the influence of the water. If preparations are examined in glycerine, the unaltered protein grains appear as highly refringent bodies, which at first sight look like small starch grains. The aleurone grains in the cells are imbedded in a protoplasmic matrix. Very beautifully developed, large protein grains, embedded in a fatty matrix, occur in the endosperm cells of the seeds of Ricinus. Sections of the tissue are easily prepared, and may

be examined in water, the disturbing action of which only takes place slowly. If, from the edge of the cover glass, we run in alcoholic solution of Iodine, the aleurone grains stain yellow: they give, in fact, proteid reactions. If sections lying in water are treated with alcohol from the edge of the cover-glass, the crystalloids in the aleurone grains become fairly distinct. On examination of sections in a drop of anhydrous Acetic acid (glacial Acetic acid) the protein grains swell up considerably, the crystalloids swell and disappear, but the globoids stand out sharply.

Sections of the endosperm of seeds of Bertholletia excelsa (Brazil nut) are particularly interesting. If absolute alcohol be added to a section lying in water, the characteristic enclosures of the aleurone grains come clearly into view (for illustrations see Pfeffer in Pringsheim's *Jahrb. f. wissenschl. Botanik*, Bd. 8, Tafel 36, Figs. 16 and 17). We have here firstly the proteid crystalloids, which in Bertholletia are comparatively large, and then the globoids, compounds of a double phosphate of Calcium and Magnesium. If we treat sections of Brazil nut with 1 per cent. solution of Osmic acid (aqueous solution of the acid, which must be kept in the dark), we shall observe the crystalloids still more distinctly, since they only slowly become yellow, while the rest of the cell contents, and especially the

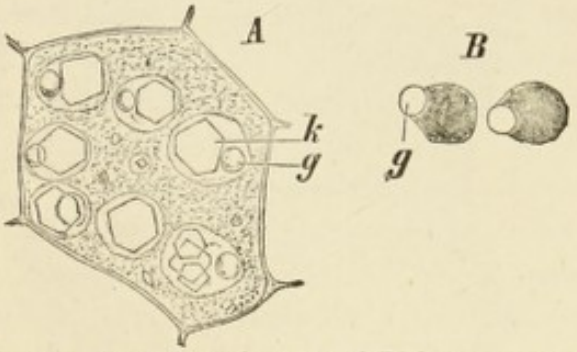

FIG. 40.—From the endosperm of Ricinus communis. *A*, an endosperm cell with its contents in water; *B*, single aleurone grains in olive oil. *g*, the globoid; *k*, the crystalloid. Magn. 540. (After Strasburger.)

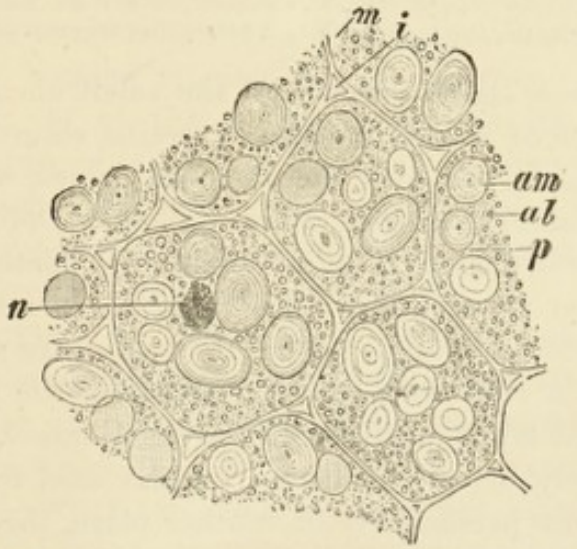

FIG. 41.—Cells from the cotyledons of the pea. *m*, cell wall; *i*, intercellular space; *am*, starch grains; *al*, aleurone grains; *p*, ground substance; *n*, cell nucleus, drawn in after treating the preparation with methyl green Acetic acid. Magn. 240. (After Strasburger.)

fatty ground substance in which the aleurone grains lie, rapidly assumes a dark colour. In the aleurone grains of Ricinus we can similarly prove the existence of proteid crystalloids by examining delicate sections of the endosperm in Osmic acid (see Fig. 40).

We prepare delicate transverse sections from the cotyledon of a ripe pea. On the cut surface we place a drop of glycerine, and examine the section in glycerine, diluted with about one-third its volume of distilled water. The microscopic structure which we observe is depicted in Fig. 41. We see roundish cells with triangular intercellular spaces between them. In the cells we find a very finely granular matrix. In this are embedded the fairly large starch grains and the small aleurone grains. On the addition of Iodine, the starch grains stain blue; but the ground substance, and also the aleurone grains, which consist essentially of proteid material, stain yellow. Delicate sections through the cotyledons of a pea, stained with methyl green Acetic acid, show that in each cell there is present a nucleus, which has become greenish blue in colour. If we prepare a delicate transverse section of a ripe wheat grain, moistening the cut surface with glycerine, and lay it in glycerine for examination, we shall find that immediately below the fruit coat and seed coat, which we shall discuss more particularly in another place, there is a layer of rectangular cells. No starch grains are present in the cells, but they contain many small aleurone grains. The cells of the more deeply lying tissue contain large quantities of starch (see Fig. 42).

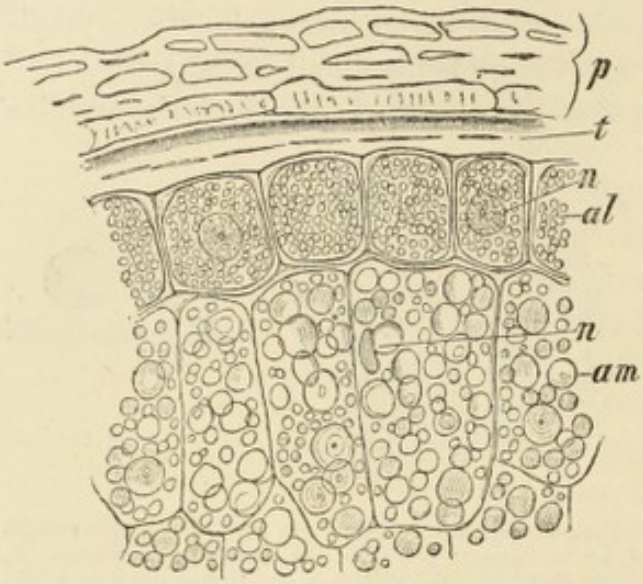

FIG. 42.—Transverse section of a wheat grain (Triticum vulgare). *p*, fruit coat; *t*, seed coat. In the adjacent endosperm cells, *al*, aleurone grains; *am*, starch grains; *n*, cell nucleus. Magn. 240. (After Strasburger.)

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[1] See Kienitz-Gerloff, *Botan. Zeitung*, 1891, where the literature is given. See also Strasburger's *Practical Botany*.

[2] See Detmer, *Lehrbuch d. Pflanzenphysiologie*, 1883, p. 151.

[3] See Loew and Bokorny, *Die chemische Ursache des Lebens*, and *Botan. Zeitung*, 1882, p. 824.

[4] See Schimper, *Botan. Zeitung*, 1880, No. 52.

II. DISORGANISATION OF THE MOLECULAR STRUCTURE.

47. The Influence of Low Temperatures on Plants.

Plants behave very differently under the influence of low temperatures. Many plants (many lichens, mosses and bacteria, but also higher plants, *e.g.* Bellis perennis and Stellaria media, etc.) keep alive when frozen at a temperature of from -6 to $-8°$ C., and then quickly thawed. I placed leaves of Primula elatior in winter in glasses, which were closed and then surrounded by a freezing mixture consisting of snow and common salt. The leaves remained for six hours exposed to a temperature of -5 to $-8°$ C., and were then quickly thawed by immersion in water at a temperature of 6° C. At the close of the experiment, the leaves were still living.

If we expose potatoes in the open, or in glass vessels surrounded by a freezing mixture, to a temperature of $-8°$ C., they freeze through and through, and become ringing hard. Leaves, *e.g.* leaves of the Crassulaceæ, or of the cabbage, rape, or bean, when exposed to a temperature of $-8°$ C., freeze, and become as brittle as glass. If we thaw the frozen potatoes or leaves by placing them in water, they perish. They have frozen to death, and now exhibit the characteristics to be described in 48.

I have fully satisfied myself by repeated experiments that potatoes whose tissues have been really frozen, always prove, after thawing, to be dead, whether they are thawed slowly or quickly. We place a few potatoes in water in a large vessel, and expose this to a temperature of $-8°$ C., so that the potatoes slowly freeze. The ice and the potatoes can then be very slowly thawed by placing the vessel in a place kept at a temperature of $+1°$ $-+2°$ C. The thawed tubers are dead. I obtained the same result with leaves of Escheveria. I have also experimented with plants of Zannichellia palustris, which, when lying in water, exposed to the light, gave off large quanties of Oxygen. After

being frozen with the water, and subsequently thawed out, they were found to be dead, and no longer exhibited assimilatory activity.

I have found it very instructive to expose leaves of Begonia manicata, either in the open or indoors, to a temperature of $-5°$ C. or $-10°$ C., placing them under a bell-glass, with their stalks dipping into water, but with their blades exposed to the air. In freezing, the leaves become discoloured, and the discoloration does not disappear on thawing. Exposure to the low temperature causes disorganisation of the protoplasm, so that the acid cell-sap is able to act on the chlorophyll bodies, and destroys their pigment. If we examine microscopically surface sections of leaves of Begonia manicata which have been killed by freezing, it is in fact seen that the chlorophyll bodies are not, as normally, green, but yellow in colour. Experiments with these leaves are instructive, since the change of colour appearing on the reduction of temperature indicates directly that the mere freezing brings about the death of their cells.[1]

It is further an important fact determined by various physiologists, that the same structures which suffer if frozen when rich in water, are not injured by exposure to cold when poor in water.[2] We may readily prove that this is the case by investigating air-dry seeds and soaked seeds respectively of Phaseolus, Pisum, Triticum, etc. For example, I have taken on the one hand air-dry wheat grains, and on the other wheat grains which had been lying in water for seven hours, and exposed both for fifteen hours to a temperature of $-10°$ C. in small glasses. I found that the former, when placed on moist sand under normal conditions for germination, are still capable of germinating, while the latter do not germinate and perish.[3]

All investigations clearly bring out this fact, that different plant structures, and the same structures in different conditions, are by no means equally sensitive to the influence of low temperatures.

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[1] See Detmer, *Botan. Zeitung*, 1886, No. 30.

[2] See Detmer, *Vergleichende Physiologie d. Keimungsprocesses d. Samen*, 1880, p. 392.

[3] Further literature: Sachs, *Versuchsstationen*, 1860, *Berichte d. sächs. Gesellschaft d. Wiss.*, 1860, Bd. 12, p. 27, and *Flora*, 1862; Göppert, *Wärmeentwickelung in der Pflanze*, 1830.

48. The Changes Which Take Place in Plants on Freezing.

When plant structures are frozen, there is by no means, as experience teaches, any rupture of the cell-membrane. If, *e.g.*, we allow filaments of Spirogyra to freeze in a drop of water on a slide, no breaks are to be perceived in the cell-walls after thawing. We know further indeed that in the freezing of tissues, the formation of ice takes place as a rule only in the intercellular spaces, etc., and not in the cells themselves.

Death from freezing is certainly to be referred to a breaking down of the molecular structure of the protoplasm, as immediately follows from the fact that, when the cells have been killed by freezing, the protoplasm has lost its normal impermeability to colouring matters, acids, etc.

If we lay frozen pieces of beetroot in water at the ordinary temperature, it takes up the red colouring matter in large quantities, whereas the sap does not escape from the cells of unfrozen pieces of beetroot when laid in water after being rinsed. Frozen potato tubers after being thawed give up large quantities of sap under slight pressure. The cells, owing to disorganisation of the protoplasm, have lost their turgor, like those of frozen leaves, which hang down limp, and rapidly dry up, as is very well seen in frozen leaves of Begonia or Escheveria.

If starch paste is frozen, and then thawed, we no longer have before us a homogeneous fluid, but a spongy mass, the pores of which are filled with fluid. Here obviously a rearrangement of the molecules has taken place, and the experiment serves to illustrate to us in some measure some of the processes which take place in the protoplasm of plant cells in freezing.

49. Formation of Ice in Freezing Plants.

A slice of beetroot, a few centimetres thick, well washed and then wiped dry, is placed in a dish, which is covered with a glass plate to prevent evaporation of water, and exposed to a temperature of say $-6°$ C. When the piece of root is completely frozen, we find that its surface is covered with a crust of ice, which, if properly examined under the microscope at a temperature below $0°$ C., is seen to consist of rods of ice arranged parallel to one another. The ice is particularly abundant on the under side of the slice of root, *i.e.* where it has been in contact with the bottom of the dish. This ice is not coloured red, from which it is clear

that not cell-sap but almost pure water has frozen out of the cells.

Under certain conditions, it is true, ice can form within the cells of freezing plant structures, but usually the water passes over into the intercellular spaces, or other cavities in the tissue, and freezes there. We cut off the upper part of a large beetroot, and fix it in place again with thread, after scooping out not too large a hole in the lower piece. If we now for some time expose the root to a temperature of say $-8°$ C., we shall find that considerable quantities of ice accumulate in the cavity.[1]

To prove that in freezing plants the first formation of ice takes place in the intercellular spaces, sections of frozen potatoes or carrots, cut with a very cold knife, are laid on a well-cooled object glass, and observed under the microscope while slowly thawing. We see that the ice crystals have formed not in, but between, the cells, and that the cell rows which were forced asunder by the ice, naturally no longer come into complete contact when the ice melts. Therefore when plant structures freeze, the cells give off water. This next appears in the intercellular spaces as ice, and as the masses of ice grow, the intercellular spaces also increase in size.

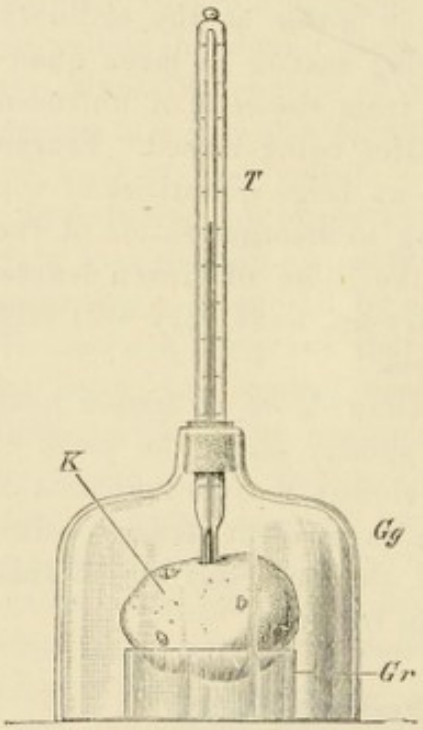

FIG. 43.—Apparatus for investigating the changes of temperature in freezing potatoes.

It is very instructive to investigate the changes of temperature taking place within plant structures during the process of freezing, as was first done by Müller-Thurgau. I have employed for this purpose the arrangement represented in Fig. 43. Under a tubulated bell-glass, *Gg*, lies the glass ring, *Gr*. On this is placed the object to be examined, say a potato, *K*. By means of a cork borer, we make a hole in the potato reaching to its middle, and, after drying the hole with blotting paper, insert in it the cylindrical bulb of a sensitive mercury thermometer, *T*, graduated to tenths of a degree. It is very desirable for the bell-glass to be provided with two tubulures, the second serving for the reception of an additional thermometer to indicate the temperature of the

air in the neighbourhood of the tuber. The whole apparatus is placed in a large dish, and the investigation is carried out in a cold room. We surround the bell-glass in the dish with a freezing mixture (snow and salt), and then read off every five minutes the position of the mercury in the thermometers. The temperature of the potato gradually sinks to $-3°$ or $-4°$ C. Suddenly however it rises again to $-1°$ C., remains for some time fairly constant at this point, and then again sinks till the temperature of the air surrounding it, *e.g.* $-8°$ C., is reached. When potatoes are exposed to a temperature below 0° C., they first of all become supercooled, without any formation of ice taking place in the tissue. When the maximum of supercooling has been reached, formation of ice suddenly sets in, and, through the consequent liberation of heat, the temperature of the potato rises to its freezing point, which is situated at about $-1°$ C. The temperature of the tubers then gradually sinks to that of the surrounding medium. Other plant structures behave in a similar manner. Thus, *e.g.*, I wrapped round the bulb of a mercury thermometer a strip of a Begonia leaf (B. manicata), fastened it with thread, and then cooled it. The maximum of supercooling lay at $-4{\cdot}8°$ C., the freezing point at $-0.8°$ C. Not until this temperature was reached did the strip of leaf become discoloured (see 47).

For exhaustive researches on the changes of temperature in freezing plant structures we may employ a freezing chamber of the following construction (Müller-Thurgau). It consists of a cubical wooden box, with sides 1 m. long, and with double bottom and sides packed with dry sawdust. Inside this box fits exactly a double-walled sheet zinc case, also open above, the inner wall of which can be lifted out. To use the apparatus the outer zinc case is first filled with pieces of ice to the level of the pillars supporting the inner case. The inner case is then put in place, and the space between the sides of the two cases is also filled with ice. If it is desired to obtain in the case a temperature below 0° C., *e.g.* $-8°$ C., we place on the top of the ice between the walls of the zinc case a certain quantity of salt, which causes a portion of the ice to melt, and so lowers the temperature. For cover we use two quadrangular trays of sheet metal, which when laid side by side cover the zinc case, but do not quite reach to the upper edge of the wooden box. Between the trays, which are also to be filled with ice to which salt is added, is left a slit, about 2 cm. wide but enlarging above, which extends across the whole case, and through

which emerge the stems of the thermometers to be used for tracing the variation in temperature of the objects, and of the air surrounding them. Finally each tray is provided with a wooden cover, which just comes up to the upper edge of the wooden box. This freezing chamber is placed in a situation where the temperature is as constant as possible, *e.g.* in a room with a north aspect. With a little attention it is then possible in winter to keep the temperature in the apparatus very constant below zero for days. In conducting the investigations the objects must be placed well in the middle of the case. The upper ends of the thermometers are fixed by suitable holders standing on one of the halves of the cover. The thermometers are graduated to tenths of a degree, and must frequently be compared with a standard instrument. Naturally they must be so constructed that the parts of the scale indicating temperatures from 0° C. to say −8° C. may rise outside the case. The readings are to be made at intervals of a minute, and clearly tabulated.

In order to grasp the fact that plant structures exposed to temperatures below 0° experience supercooling, and that their freezing point is not at 0° C., but at a lower temperature, we must recall the behaviour in freezing of salt solutions and of water retained by solid bodies by adhesion. The cells of plants, indeed, contain not pure water, but an aqueous solution of various substances, and the organised plant structures hold water. Pure water freezes ordinarily at about 0° C., but a solution (*e.g.* of common salt) always freezes at a lower temperature, and the amount of supercooling experienced before the formation of ice begins, varies with the strength of the solution. Then the temperature of the solutions suddenly rises, till the freezing point proper, which always lies below 0° C., is reached. We can easily prove these relations by suitable experiments. To investigate the behaviour, in freezing, of water retained by the force of adhesion, I have employed the method described by Müller-Thurgau. Round the bulb of a mercury thermometer was wrapped blotting paper which had been soaked with water and then externally allowed to dry. When placed under a bell-glass surrounded by a freezing mixture, the temperature of the blotting-paper gradually fell to −3° C. (the maximum of supercooling), and then suddenly rose to a point somewhat below 0° C.

The water of solutions and the water retained by solid bodies by the force of adhesion—and water occurs in plants under both

these conditions—freezes therefore not at 0° C., like pure water, but at lower temperatures. The state of equilibrium between the molecules of the water, and the molecules of the salt or those of the solid bodies, is not disturbed, so that the formation of ice can take place, till the temperature has fallen below 0° C. Certainly on formation of the ice a considerable quantity of heat is set free; but still the temperature of the salt solution, or of the solids permeated by water, does not rise quite to 0° C., since a certain quantity of heat is employed in separating the molecules of water from the molecules of salt, or from the molecules of the solid bodies, as the case may be.

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[1] On this subject and what follows see Müller-Thurgau, *Landwirthschaftl. Jahrbücher*, Bd. 9, p. 133.

50. Death resulting from Exposure to too High a Temperature.

We first employ young plants of Zea, Nicotiana, Cucurbita, Phaseolus, or Tropæolum, growing in small flower pots. The plants are suitable for the experiments as soon as a few leaves have unfolded. We then warm the air under the bell of a thermostat to the temperature whose effect is to be studied, and when the temperature has become constant at this level, one of the plants is introduced. We wait until the desired temperature is again reached within the apparatus, and then leave the plant exposed to it for a certain time. It is often desirable, in order to obtain more rapid adjustment of temperature, to water the soil in the pots with warm water. One thermometer is placed in the soil in which the plant is rooted; another is suspended within the bell-glass so as to touch the aerial parts of the plant. We can now vary the experiments in many ways. We may, *e.g.*, leave our research plants for half an hour in the apparatus with the air at a temperature of 40° to 45° C., or we may expose them for 10–30 minutes to a temperature of 52° C. We then remove the plants from the thermostat, expose them to normal conditions of environment, and observe their further behaviour. Usually they may be kept for half an hour in air at a temperature of 40° C. without injury, but exposure for a period of from 10 minutes to half an hour to a temperature of 52° C. as a rule kills them. It must be observed, however, that the plants do not by any means immediately die off after exposure to too high a temperature, *e.g.*

52° C. for 10 minutes. On the contrary, death may not take place for days. The newly matured leaves gradually become discoloured, while the older leaves, the internodes and also the buds do not perish till later. In carrying out exact comparative investigations as to the influence of high temperatures on plants, it is necessary to repeat the experiment at any particular temperature several times, using also each time a fresh and perfectly normal plant. In this way we avoid errors. It would also be instructive to leave plants in the thermostat for a longer period (several hours or even days) at comparatively low temperatures, *e.g.* 35–40° C., and observe their subsequent behaviour.

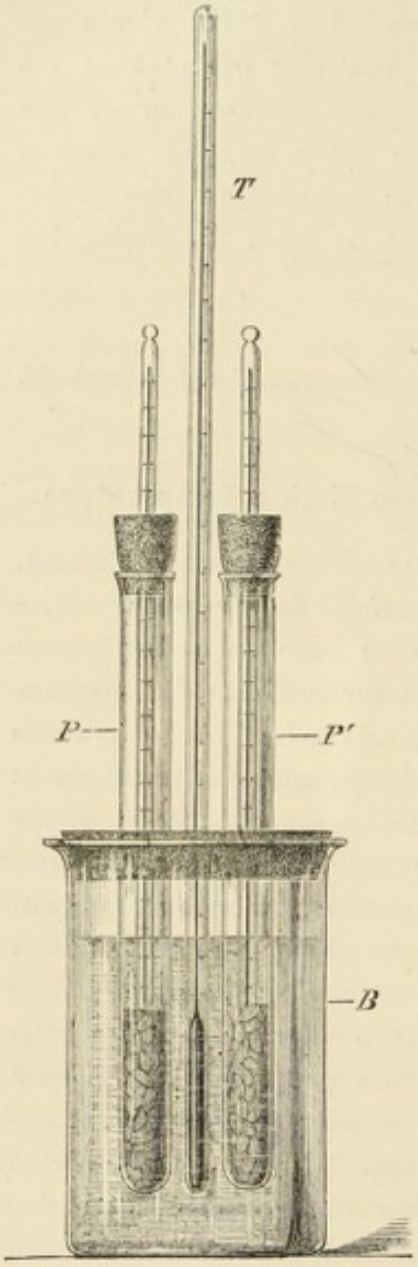

FIG. 44.—Apparatus for investigating the influence of high temperature on seeds.

Plants suffer if exposed for ten minutes, or somewhat longer, to a temperature of 52° C. in air, but exposure for ten minutes to temperatures of from 45–48° C. causes their death, if their environment is water instead of air. To demonstrate this fact, we invert a potted plant (laying strips of wood over the top of the pot to prevent the soil from falling out), and plunge the aerial part of the plant into water at the temperature in question. The temperature is kept constant during the experiment, and at the end of ten minutes we remove the plant from the water, and observe its further behaviour.

In studying the influence on plants of water at high temperatures, we may also experiment with organs severed from the plant, *e.g.* leaves. I have used, *e.g.*, leaves of Begonia manicata and Vitis vinifera, which are very suitable because they undergo a marked change in colour when their cells die. If we immerse leaves of Begonia for fifteen minutes in water at a temperature of 40° C., their cells do not die. When

immersed in water at 75° C., the leaves almost immediately become discoloured, and are then dead. Water at 55° C. kills the leaves within two minutes.

To demonstrate the important fact that plant structures, especially seeds, bear exposure to high temperature far better when dry than when saturated with water, we use the apparatus depicted in Fig. 44. The beaker *B* is filled with water. Through the large cork with which it is closed pass the thermometer *T*, and the two test-tubes *P*, *P'*. These last are fitted with corks, through which also pass thermometers. The whole arrangement is supported on a ring stand, and dips into the water of a water-bath. We now heat with a gas or spirit flame, till the thermometers in the test-tubes indicate the temperature at which we wish to experiment (*e.g.* 50° C., 60° C., or 70° C.). Air-dry seeds (50–100) are now placed in one of the test-tubes, soaked seeds in the other. We use seeds of Pisum, Zea, or Triticum, and leave them in the apparatus for some time (say an hour), keeping the temperature constant. The seeds are then laid in sawdust, and exposed to normal conditions for germination. The air-dry seeds still prove in part capable of germinating; the soaked seeds all perish, and do not germinate.

Air-dry grains of Pisum, Zea, or Triticum may be exposed for an hour to temperatures of 65° C. or 70° C., without all of them losing their power of germinating, but on the other hand, their ability to germinate is certainly more or less diminished. I exposed air-dry grains of wheat for an hour to a temperature of 62° C., and a fairly large percentage of them were found to be still capable of germination, but soaked seeds similarly exposed for an hour to a temperature of 62° C. all perished.[1]

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[1] Literature: Sachs, *Flora*, 1864, p. 5, and *Handbuch d. Experimentalphysiologie der Pflanzen*, 1865, p. 64. Further see Detmer, *Vergleichende Physiologie d. Keimungsprocesses d. Samen*, 1880, p. 401; Höhnel in Fr. Haberlandt's *Untersuchungen auf dem Gebiete des Pflanzenbaues*, Bd. 2, p. 77, and Detmer, *Botan. Zeitung*, 1886, No. 30.

51. Changes experienced by Plants in Death caused by Exposure to too High Temperatures.

We take a young bud leaf of Elodea canadensis, and after ascertaining that the protoplasm of its cells is in active movement, immerse it for a short time (say one minute) in water at a tem-

perature of 60° C. Owing to the influence of the high temperature, the movement of the protoplasm is at once arrested, and is not resumed with lapse of time. The leaf becomes disorganised, and is in fact dead.

Very many kinds of leaves (I have used, *e.g.*, cabbage leaves) do not change much in colour, when placed for a time in water at a high temperature (*e.g.* 60° C.). The leaves, however, very quickly die; the cells lose their turgidity, and the leaves become flaccid. Nor can they be restored to their normal turgescent condition. If we immerse in hot water leaves whose cells are rich in acid (I found leaves of Begonia manicata and Vitis vinifera very satisfactory), they rapidly become discoloured, since the chlorophyll grains, owing to the destruction of the protoplasm, are now brought into direct contact with the acid cell-sap, and the acids decompose the chlorophyll. If we lay Begonia leaves in water at a temperature of 55° C., they become discoloured in the course of two minutes; in water at a temperature of 75° C., they lose their green colour almost instantaneously. Microscopic examination of tangential sections of killed leaves of Begonia manicata shows that the chlorophyll granules are no longer green but brownish in colour.

That the protoplasm, in death brought on by exposure to too high temperature, loses its normal constitution, and consequently allows the acids of the cell-sap to traverse it easily by osmosis, we can easily determine, according to my experience, as follows:—A piece of the petiole of a living Begonia manicata leaf is washed, and laid in distilled water. A second piece of the same petiole is killed by immersion in water at 60° C. When it has become discoloured, we at once place it in distilled water. After a time we remove the pieces of leaf, and add to the water in each case Calcium chloride solution. That in which we laid the killed piece of leaf becomes turbid, owing to separation of Calcium oxalate; the other remains clear. The disorganised protoplasm has become permeable to the Oxalic acid of the cell-sap, and has allowed it to pass out into the surrounding water.

If hairs from the stamens of a Tradescantia flower are killed by immersion in water at 55° or 60° C., then mounted on a slide in a drop of water, and examined under the microscope, it is seen that the red or violet pigment of the cell-sap passes over into the space between the protoplasm and the cell-wall, and ultimately escapes from the cells into the surrounding water. The uninjured

protoplasm is impermeable to the pigment. If we carefully rinse pieces of fresh beetroot in order to remove the sap coming from the cut cells, and then lay them in water, they yield no colouring matter, even after remaining in the fluid for an hour or more. If, however, the cells are first killed by heat, and then laid in water at the ordinary temperature, their colouring matter rapidly escapes.

Pieces of turnip are killed by immersion in water at 60° C., and then laid in beetroot juice, together with pieces consisting of living cells. The former will be found within twenty-four hours to be coloured red through and through, while the pigment has not penetrated the latter.

52. The Effect of Mechanical Injury.

Although it is known to everybody that plant structures can survive slight pressure or slight tension without injury, but suffer destruction of their molecular constitution if submitted to considerable mechanical injury, a few not quite unimportant experiments may nevertheless be made which clearly bring out this last fact.

Water at 15–20° C. is poured over some potato starch. An equal quantity of starch is mixed with clean quartz sand, and ground down as thoroughly as possible in a mortar. Water is then poured over this also. After a few hours we filter off the fluids. Testing with Iodine, we find that the water has extracted granulose from the starch ground down with sand, but we can detect no granulose in the other case. The mechanical injury has destroyed the molecular organisation of the starch grains, which consequently give up granulose to the fluid, while the uninjured grains cannot do so.

If we violently squeeze between the fingers the blade of a leaf of Begonia manicata, the injured parts at once become brownish in colour. Microscopic examination of tangential sections of the crushed portion teaches that the chlorophyll grains, which in the normal cells are beautifully green, have become discoloured. The pressure has destroyed the protoplasmic constituents of the cells. They have become permeable to the acid cell-sap, and this has caused the decomposition of the chlorophyll.[1]

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[1] See Detmer, *Botan. Zeitung*, 1886, No. 30.

53. The Effect of Desiccation on Plant Structures.

If cabbage sprouts are cut and kept without water, they soon wither. If the process has gone far, we cannot bring the shoots back to their normal condition by supplying them with water; but shoots which have just begun to wither, will often revive again if plentifully supplied with water.

To familiarise ourselves with the effect of desiccation on seeds and seedlings, we employ wheat or pea seeds. Part of the material is soaked for twenty-four hours in water, and then at once placed in glass dishes and left exposed to the air to dry. The rest is similarly exposed to the drying influence of the air when the rootlets have just emerged, or have more or less developed. When the seeds and seedlings have become air dry, we lay them in moist sawdust, and observe their behaviour. The soaked seeds have suffered little, and this is also the case with the seedlings whose roots have only developed to a very small extent. The influence of the desiccation on somewhat further advanced seedlings is shown in the death of the young parts; but on renewal of the water supply these are replaced by the formation of adventitious roots and the development of previously existing axillary buds. Still more advanced seedlings usually perish completely.[1]

If clumps of Barbula muralis are dried for several weeks in the air, or over Sulphuric acid in a desiccator, and then moistened and placed on moist earth, they go on growing again without losing the old leaves. Many other mosses behave in a similar manner, but others prove to be very sensitive to desiccation.[2]

I conducted a series of observations to determine how seedlings, more or less dried, but still fairly rich in water, behave as regards energy of respiration, as compared with normal ones.[3] About thirty pea seedlings were examined with respect to their respiration in the manner indicated in the Third Section. The amount of Carbon dioxide expired by the seedlings in two to three hours at a constant temperature is determined. Then they are deprived of water for some days, and the more or less dried material is again examined at the same temperature as before. Its respiratory energy is found to have fallen considerably. My observations show that air-dry seeds do not give off a recognisable quantity of Carbon dioxide.

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[1] See Nowoczek in Haberlandt's *Wissensch.-prakt. Unters. auf dem Gebiete d. Pflanzenbaues*, Bd. 1, p. 122.

[2] See Schröder, *Untersuchungen aus dem botan. Institut zu Tübingen*, Bd. 2, p. 18.

[3] See Detmer, *Landwirthschaftl. Jahrbücher*, Bd. 11, p. 230.

54. The Action of Electricity on Plants.

Comparatively little is as yet known concerning the action of electricity on plants, especially as regards the finer details.[1] The chief fact of physiological interest is that constant currents, as also induction currents, are not without influence on the movements of the protoplasm, since they usually retard or completely arrest the movements, and ultimately cause the death of the cells.

To study the phenomena in question, we employ young leaves of Elodea, or hairs from the younger parts of a vegetable marrow. We mount the objects in a drop of water, on a slide of the form indicated in Fig. 45. On the glass slip *G* are cemented by means of asphalte varnish (a solution of asphalte in oil of turpentine) two brass plates, *M*, *M*, to each of which is soldered a binding screw. The two strips of tinfoil, *St*, *St*, are attached to the brass plates, and to the plate of glass, by means of asphalte varnish. A space is left between them, and here is placed the drop of water in which the object to be examined is mounted. To study the influence of induction currents upon the plant cells, we connect up the ends of the wires from the induction apparatus by means of the binding screws, and can then make microscopical observations while the currents are acting upon the cells. It is important in physiological experiments to be able to regulate the strength of the current, and for this purpose we employ an induction apparatus such as is employed for medical purposes. The apparatus is usually supplied in a suitable case, together with the current-generating element. The regulation of the strength of the current may be effected by means of du Bois Reymond's sliding apparatus (see Fig. 46), in using which we connect up the ends of the primary coil *A* with the current generator and the Rheotome, while the secondary coil *B* is joined up with the

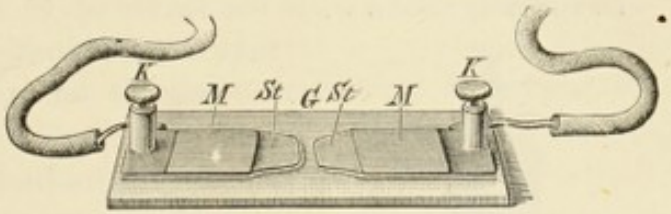

Fig. 45.—Object-glass for investigating the action of electric currents on plant structures.

binding screws of the slide. The Rheotome (generally a magnetic hammer) with which the primary coil is usually provided is not represented in Fig. 46. For the generating element we may employ the Bichromate cell represented in Fig. 47.

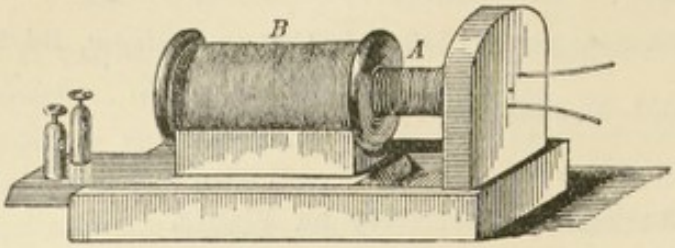

FIG. 46.—Induction apparatus.

The bottle contains a solution prepared by gradually adding 92 gr. of powdered Potassium bichromate to 93·5 c.c. of concentrated Sulphuric acid, and dissolving the mass in 900 c.c. of water. The zinc plate can be lowered between the carbon plates, or, when not in use, raised out of the fluid by means of the rod carrying the binding screw.

In investigations with the leaves of Elodea canadensis, I found that weak induction currents arrested the movements of the protoplasm of the cells; on cessation of the current, the movement gradually began anew. Strong induction currents permanently arrested the protoplasmic movements of a cell. Since stronger currents kill the cells, and the death of the protoplasm is readily indicated by the fact that it becomes permeable to many substances (*e.g.* pigments) which it does not allow to traverse it when living, it would be instructive to allow electric stimuli to act on hairs from the staminal filaments of Tradescantia. The death of the cells would be at once demonstrated by the escape of the pigment from the cell-sap and from the cells. In studying the effect of electric currents on plant cells, we notice also the changes of form that the protoplasm suffers under the influence of the current.* It is further instructive to make the following experiment:[2]—We lay on a sheet of glass two strips, each a few centimetres in length, from a leaf of Begonia manicata. Through one piece of leaf we now

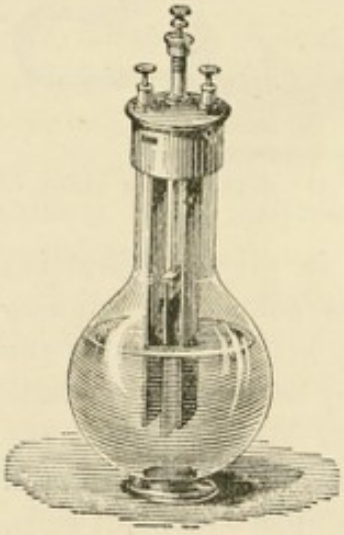
FIG. 47.—Bichromate cell.

* In investigating the influence of electricity on plants, we must further take care to use unpolarisable electrodes, or the results will be vitiated. Respecting these, see 63.

pass a not too weak induction current for about fifteen minutes, placing one electrode (a small piece of metal) on each end of it. The second strip we use merely for the purpose of comparison. At the end of the time both pieces of leaf are placed in a closed glass. The control strip remains green and fresh, the one experimented upon quickly becomes brown, loses its turgidity, and gets flaccid, since the induction current has killed the protoplasm of its cells.

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[1] For the literature, see scattered references in Pfeffer's *Handbuch der Pflanzenphysiologie.*

[2] See Detmer, *Botan. Zeitung*, 1886, No. 30.

55. The Action of Poisons on Plants.

In making experiments to prove whether a particular substance has a poisonous effect on the cells of plants, we may work with seeds. It must always be remembered that a substance which injures or destroys one kind of plant must not, therefore, be considered poisonous to all plants. To test the substances we prepare solutions of definite strength, 0·1 p.c., 0·2 p.c., 0·5 p.c., 1·0 p.c., or weaker or stronger ones as may be desired (using, *e.g.*, corrosive sublimate, Copper sulphate, Salicylic acid, Carbolic acid, Citric acid, atropin, chloride of quinine, common salt, etc.). These solutions we pour into small glasses, and then introduce a fair number of pea seeds or wheat grains. After twenty-four hours the soaked seeds are placed in water in shallow dishes, or laid in damp sawdust. We now note how many seeds germinate in a particular time, and what length the parts of the seedlings attain, as compared with those seedlings which have developed from the first under perfectly normal conditions. In this way I found, *e.g.*, that even 0·1 p.c. solutions of Salicylic acid had an extremely poisonous effect on pea seeds.[1]

We further cultivate seedlings of Pisum in flat dishes, taking care that the cotyledons are always about half covered with water. After a few days we determine the length of the roots and stems of the seedlings, replace the water by solutions of various substances of known concentration, and leave the seedlings in these for twenty-four hours. We then again measure the length of root and stem, put the seedlings back into pure water, and determine whether or not they grow. I find that many poisons permanently arrest the growth of seedlings; others also certainly

arrest the growth, but it is resumed when the seedlings are subsequently placed in distilled water.

I have also placed soaked seeds of Pisum sativum in water, in flat glass dishes, near which was placed another dish containing chloroform, the whole being then covered with a bell-glass. At a moderate temperature (18° C.) not a single seed germinated.[2] We can readily demonstrate in lecture the poisonous effect of chloroform on plant cells, by pouring some chloroform into a flat dish and placing this under a bell-glass together with a leaf of Begonia manicata, the leaf-stalk of which dips into water (see Fig. 48). The leaf becomes discoloured, because the protoplasm of the chlorophyll grains is killed, and hence becomes permeable to the acids of the cell-sap, which can now decompose the chlorophyll.[3]

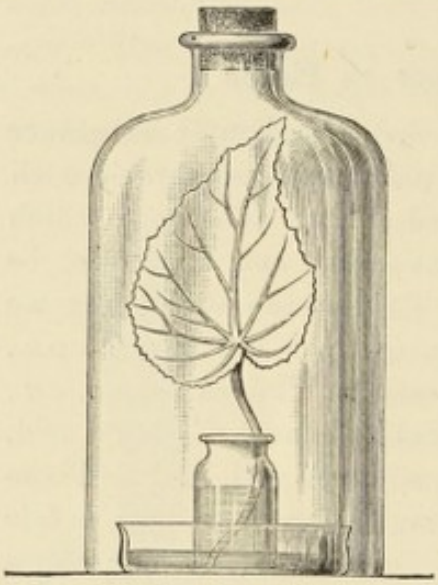

FIG. 48.—Apparatus for investigating the action of chloroform on plant structures.

In order to ascertain whether particular substances prejudice or completely arrest the development of Penicillium or of bacteria, we conduct cultures in the manner indicated in 35 and 38, but adding to the food solutions definite quantities of the substances (*e.g.* corrosive sublimate, Salicylic acid, etc.) whose effect on the fungi is to be tested. Control experiments, in which the poisons are not added to the solutions, will, of course, be necessary.

Thus, for example, one lot of Pasteur's food solution containing cane-sugar (for composition of the solution, see 18), after being put aside for eight days, had become very turbid owing to copious development of bacteria, while another portion of similar composition, except that some 0·2 p.c. Salicylic acid had been added to it, remained clear.

To ascertain the influence of poisons on Spirogyra or other algæ, we place single filaments of the plants in the solutions, *e.g.* solutions of Copper sulphate, Oxalic acid, etc.[4] If the solutions are not too strong, so that the poisonous action proceeds slowly, the first effect is generally a shrinking of the nucleus and swelling of the chlorophyll bodies.

[1] See Detmer, *Landwirthschl. Jahrbücher*, Bd. 10, p. 733.

[2] See Detmer, Wollny's *Forschungen auf dem Gebiete der Agriculturphysik*, Bd. 5, p. 253.

[3] See Detmer, *Botan. Zeitung*, 1886, No. 30.

[4] See Loew, *Flora*, 1892, H. 3, pp. 374 and 386.

III. MOLECULAR PROCESSES IN PLANTS.

56. Imbibition.

We prepare a delicate transverse section through the stem of a young Laminaria. On examination of the section in alcohol, but little is to be seen of the details of structure. They stand out clearly, however, on addition of water. We distinguish an outer cortex, the cells of which possess brown membranes, and the so-called inner cortex which forms the main mass of the tissue, and of which the cell-membranes are colourless. In the middle of the section we observe the medullary tissue, consisting of tubular cells. If we mount sections of Laminaria in alcohol, and then introduce water from the edge of the cover-glass, we can readily determine by microscopic examination that at the moment of absorption of water the sections increase considerably in volume. We can also prove the increase in volume by taking the dimensions of a piece of Laminaria stalk with a millimetre scale, on the one hand when dry, and on the other after saturation with water. The substance of Laminaria is therefore capable of swelling by absorption of water, and the process by which this is brought about is termed Imbibition. The increase in volume of pieces of Laminaria placed in contact with water does not, however, proceed indefinitely, but is limited in extent, and this is of considerable importance, because it teaches that a piece of Laminaria—and organised plant structures in general behave in the same way—when placed in water at the ordinary temperature behaves very differently from, say, gum.

If a piece of a Laminaria blade, whose weight in the dry state is known, is laid in water, and after lapse of definite intervals of time (*e.g.* every eight minutes) removed from it, dried with blotting-paper, and weighed, it will be found that the absorption of water by the object during such periods is at first rapid, gradually diminishes, and finally ceases. If we suspend the soaked piece of Laminaria in the air by means of platinum wire, and

determine its weight from time to time (say every half-hour), we shall find that at first a large quantity of water evaporates, and then gradually less and less in each period.

A few skinned peas are placed in water at about 5° C. Others, as nearly as possible of the same weight, are laid in water at about 20° C. At the end of four hours the seeds are dried and again weighed. It appears that the seeds have taken up more water at the higher than at the lower temperature; elevation of temperature accelerates therefore the process of imbibition. Some skinned peas are placed in water, and an equal quantity in 10 or 20 p.c. solution of common salt. It is easily ascertained by weighing, at the end of a few hours, that imbibition does not proceed so rapidly in salt-solutions as in pure water.

To investigate the increase of volume experienced in imbibition by seeds, or cubes of wood from different plants, we first place the material in the dry state in a narrow glass cylinder, the volume of which, up to the level of a mark made near the top, is accurately known. We now run diluted alcohol into the cylinder from a burette till it reaches the mark, and knowing the volume of alcohol required, we can at once calculate the volume of material used. In determining the volume of the soaked seeds or cubes of wood, we proceed in the same manner, but use water instead of alcohol. By comparative investigations as to the increase in weight and volume which plant structures experience in imbibition, we shall often be able to prove, especially in experiments with woods, that the actual increase in volume observed by no means corresponds with the volume of water absorbed. This fact is, moreover, quite intelligible if we bear in mind that only the water imbibed by the solid wood substance causes increase in volume; the mere filling of the lumina of the wood elements with water cannot bring about any increase in the volume of the material. Experiments in which we determine at the same time the increase in weight and volume of one and the same piece of wood during absorption are especially instructive, inasmuch as they prove beyond doubt that imbibition is by no means comparable with capillarity. When fluids enter a body by capillary attraction, they always penetrate into previously existing spaces, and therefore capillarity does not give rise to any increase in volume in the fluid-absorbing bodies. When imbibition takes place, the molecules of fluid penetrate between the micellæ of the bodies; they actually make space for themselves between

the micellæ, and in this way is brought about the increase in volume of the bodies.

Wood when placed in water swells much more considerably radially and circumferentially than in the direction of the axis. We can easily prove that this is the case if, with a millimetre scale, we take the measurements of fairly large cylindrical pieces of wood, respectively when dry and when soaked with water. We use for the experiment pieces of wood about 100 mm. long and 80 mm. in diameter.

Very energetic imbibition, which finally results in the complete disorganisation of the molecular structure, can be induced, *e.g.*, in starch-grains by heat, acids, or alkalies. If potato-starch is cautiously heated on a slide over a spirit or gas flame, care being taken to replace the water lost by evaporation, a very considerable increase in the volume of the grains takes place, and at about 70° C. they become swollen up into glassy masses, whose outline it is difficult to make out.

If we mount some potato-starch in a drop of water on the slide, and very slowly run in potash or Sulphuric acid from the margin of the cover-glass, we notice that at the commencement of the action the layering of the grains becomes more distinct; but it afterwards disappears, the starch-grains undergo considerable increase in volume, and finally swell up into glassy masses.

When particles of water penetrate into bodies capable of imbibition, they must necessarily suffer condensation owing to the strong attraction which will be exerted by the micellæ upon the fluid. When, however, such condensation takes place, heat is set free, and, in fact, it can be shown that the process of imbibition is accompanied by a rise of temperature. I have placed 100 gr. of potato-starch, or 100 gr. of pea-meal, at a known temperature, in a glass cylinder, and poured over it a relatively small quantity of water of precisely the same temperature. It is best to run the water from a burette. The temperature of the mixture at once rose about 1·5° C. The rise of temperature was, however, as much as 5° C. when a little water was added to potato-starch which had been dried by warming and then allowed to cool.

In the process of imbibition, however, work also is done, both internally (in the separation of the micellæ from one another) and externally (in overcoming external resistance to the increase in volume). To demonstrate in lectures that external work is done I employ the apparatus drawn in Fig. 49.[1] Into one end

of the wooden base is screwed the wooden socket supporting a glass cylinder, *g*, 10 cm. high and 35 mm. in diameter. This cylinder receives the seeds and water. It is made water-tight at the bottom by means of sealing-wax. In the cylinder moves loosely a metallic piston, *s*, supported by a vertical piston rod, *a*, 16 cm. in length. The piston rests immediately on the seeds. The cylinder is closed by the metallic cap *k*, through the middle of which passes the piston rod, so that the cap can be freely twisted round it. The piston rod carries at the top a brass disc,

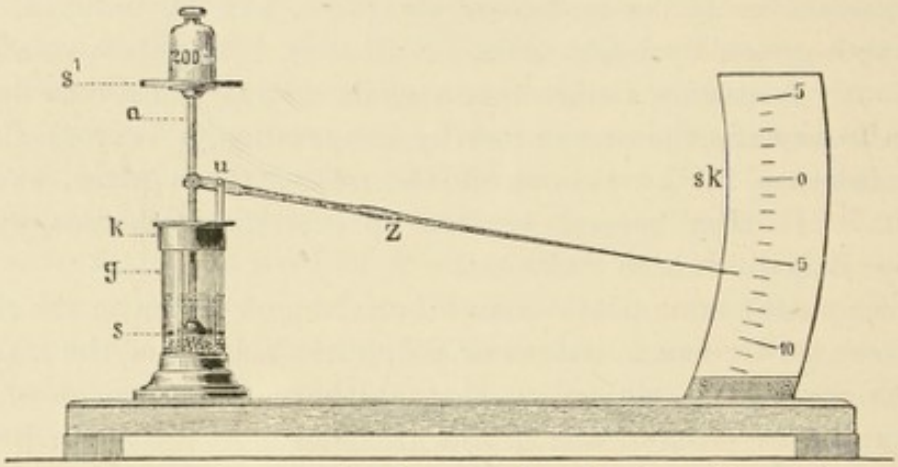

FIG. 49.—Apparatus for measuring the external work performed by swelling seeds.

s', intended to support a weight. Between the cap *k* and the disc *s'* the horizontal lever *z* is attached to the piston rod by means of a screw, so that it can be fixed at different heights. Its fulcrum is at *u*. The short arm of the lever is 2 cm. long, the long arm about 33 cm. long. The pointer reaches to the circular arc *sk* (represented on the right-hand side in the illustration), which is graduated in centimetres. Any upward movement of the piston, due to the pressure exerted by the swelling seeds, effects a downward movement of the pointer, which renders it possible to ascertain on a correspondingly magnified scale the movement of the piston resulting from the swelling of the seeds. 10 gr. of small peas placed in the apparatus were able to overcome a resistance of 1,000 gr.

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[1] Literature: Sachs, *Handbuch d. Experimentalphysiologie d. Pflanzen*, 1865, p. 431; Detmer, *Vergl. Physiologie d. Keimungsprocesses d. Samen*, 1880, pp. 78 and 290; Reinke in Hanstein's *Botanische Abhandlungen*, Bd. 4, H. 1; Schleichert, *Naturwissenschl. Wochenschrift*, Bd. 7.

57. Diffusion and Endosmosis.

If substances in solution appear at any place in the protoplasm or cell-sap of a cell, they can spread thence over the whole protoplasm or the whole cell-sap. In this process diffusion plays an important part, but the rate at which diffusion takes place is by no means always so great as we are often in the habit of thinking, and it is certainly instructive to convince ourselves of this. We place a tall glass cylinder filled with water on a table free from vibrations, drop into the water a crystal of Potassium bichromate, and cover the cylinder with a glass plate. The Potassium bichromate dissolves, but even after several days the upper layers of the fluid are only coloured faintly yellow, while the lower layers of fluid exhibit the characteristic colour of saturated solutions of the salt. In making our experiment, we have by no means excluded all the conditions which might cause currents in the fluid; and the fact accordingly stands out so much the more clearly that the distribution of dissolved substances by diffusion does not proceed with special rapidity.

This being so, any causes by which the distribution of substances dissolved in the protoplasm and in the cell-sap is accelerated, gain in importance, and among such conditions must in many cases be regarded protoplasmic currents, and movements of plants structures due to the wind.

Putting aside diffusion, osmotic processes play a most important part in the distribution of substances in the plant, and we will therefore direct our attention to them.

A glass tube about 8 cm. long and 3 cm. wide is covered at one end with a piece of pig's bladder. To make the closure perfectly tight, we first moisten the bladder, and then tie it firmly over the end of the tube with string, or better still with elastic. The tube is now completely filled with an almost concentrated solution of cane-sugar, and its upper end is closed with a rubber stopper, through which passes a long glass tube. We note the position of the cane-sugar solution in this tube, and then dip the lower end of the apparatus into distilled water. We find that the fluid at once begins to rise in the tube. The water passes by osmosis through the bladder into the sugar solution, and although a certain quantity of the sugar solution also travels in the opposite direction into the water, still the quantity of fluid flowing into the apparatus is greater than that leaving it, and the volume of fluid

in the apparatus must consequently increase. If the lower end of our apparatus is allowed to dip for a certain time (one to two hours) alternately into water at the usual room temperature, and into warm water (say at 30° C.), we can readily prove, by observing the rise of the fluid in each case, that the osmotic processes go on more actively at the higher than at the lower temperature. If the apparatus dips not into pure water, but into a 20 per cent. solution of common salt, osmosis proceeds more slowly, as may easily be determined.[1]

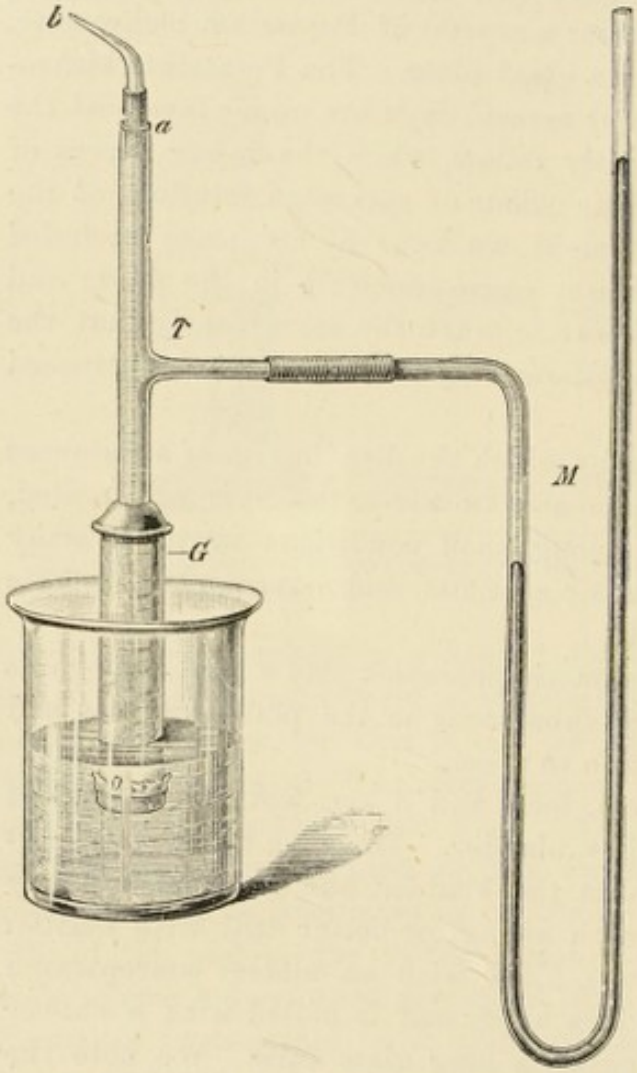

Fig. 50.—Apparatus for investigating pressure effects due to osmotic processes.

In order to understand properly many physiological phenomena, especially those caused by turgor, it is of great importance that we should convince ourselves that considerable pressures can be set up through osmotic processes. For this purpose we may use the apparatus represented in Fig. 50. The glass tube *G*, 10 cm. long and 2 cm. in diameter, is closed at the bottom with pig's bladder, and dips into water. Through the rubber stopper closing the upper end of the glass tube passes the T-shaped tube *T*, the vertical limb of which is connected at *a* with a small bent glass tube drawn out to a point at *b*. The horizontal limb of the T-tube is connected with the manometer *M* by means of thick rubber tubing, bound round with wire. The manometer contains mercury; the rest of the apparatus is completely filled with a nearly concentrated solution of cane-sugar, and the small

tube is finally fused up at *b*. The cane-sugar attracts considerable quantities of water by osmosis. A pressure is thus developed in the apparatus which causes the mercury to rise in the manometer. I found, *e.g.*, in one experiment that at the end of three days the mercury was 47 cm. higher in one limb of the manometer than in the other. The pressure in the apparatus amounted therefore to considerably more than half an atmosphere.[2]

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[1] See Detmer, *Beiträge zur Theorie des Wurzeldrucks*, in Preyer's *Sammlung physiologischer Abhandlungen*, Bd. 1, Heft 8, p. 29, Jena, 1877.

[2] For further information, see Pfeffer, *Osmotische Untersuchungen*, 1877.

58. The Diosmotic Properties of the Cell-wall and of the Protoplasm.

An excellent object in which to study the diosmotic properties of the cell-wall and of the hyaloplasm is afforded us in the staminal hairs of Tradescantia. We remove a tuft of the hairs from the filament with the forceps, and find, on submitting them to microscopical examination, that each hair consists of a single row of cells. The cell-wall, the protoplasm, the nucleus, and lastly the beautiful violet-coloured cell-sap are readily observable in each cell (see Fig. 38).

From the margin of the cover-glass we now run in glycerine, or more or less concentrated solutions of sugar or common salt. These fluids extract water from the cell-sap, and the protoplasm consequently contracts, so that spaces are formed between it and the cell-wall. We have caused the cells to pass from a turgescent into a plasmolytic condition. Our experiment further proves, however, the important fact that the hyaloplasm of the living protoplasm must be impermeable to the pigment dissolved in the cell-sap of the cells, for the colouring matter does not pass through the protoplasm with the water when plasmolysis takes place.

The effect is quite different if we allow absolute alcohol to act on the staminal hairs of Tradescantia, and thereby kill the cells. The violet cell-sap now passes over into the protoplasm, since the hyaloplasm has become permeable to the colouring matter. The protoplasm, and especially the cell-nucleus, become deeply stained, and the coloured fluid may even pass out of the cells into the surrounding medium. It is also very instructive to plasmolyse cells whose cell-sap is uncoloured, *e.g.* the epidermal cells of the leaves

of Tradescantia, with plasmolysing solutions to which pigments have been added.

I conducted investigations of this kind in the following manner: Strips of epidermis from leaves of Tradescantia were plasmolysed as above described by means of a solution of common salt, and then laid in juice obtained by crushing fairly dark-coloured cherries. The colouring matter can traverse the cell-wall; it penetrates into the space intervening between this and the protoplasm, but the protoplasm itself does not take it up. If epidermal cells of Tradescantia are first plasmolysed, then killed, by dipping the strips of epidermis in hot water, and finally laid in cherry juice, the protoplasm and the nucleus stain fairly deeply, the dead protoplasm being readily permeable to many substances which in the living condition it is unable to take up.

To prove that normally protoplasm is impermeable, but after death is permeable to sugar, we carefully rinse a few pieces of beetroot, and then transfer them to distilled water at the ordinary room temperature, some of them at once, the rest after they have been killed with hot water. After a few hours we take from the fluids small test quantities, add to them a few drops of dilute Hydrochloric acid, and boil for a short time; the presence of sugar can easily be detected by means of Fehling's solution in the fluid which has been in contact with the killed pieces of beetroot, whereas the others contain no sugar.

To prove that the normal hyaloplasm is also frequently impermeable to mineral substances, we prepare 2–4 per cent. solutions of Sodium chloride or Potassium nitrate. In these we place hairs from the stamens of Tradescantia, or strips of epidermis from the underside of the midrib of the leaf of Tradescantia discolor. The cells of T. discolor, a plant which can be obtained at any time of the year, and which must be grown under cover, have a coloured cell-sap. The solutions make their way through the cell-walls into the cells. Plasmolysis follows in the course of one to two hours, and it still persists if the objects are left in the salt solution for several hours longer. It is this which is of special importance to us, since if the protoplasm were, under the conditions described, permeable to Sodium chloride or Potassium nitrate, it would gradually, with increasing osmotic capacity of the cell-sap, swell out again in each cell.[1]

The diosmotic properties of the living hyaloplasm on the one hand and of the cell-wall on the other are, as we know, very

different. The former is usually not permeable to colouring matters, sugar, etc., while the cell-wall behaves, as regards these substances, precisely like vegetable parchment. It is therefore of interest to make some experiments which will afford us information concerning the osmotic permeability of parchment.

We may use for the dialyser a wide glass tube, closed at the bottom with parchment paper. The apparatus I have used in my experiments is constructed somewhat differently, for the sake of greater convenience in manipulation. A thick glass tube, 80 mm. long and 40 mm. in diameter, is fitted at the lower end with a brass ring, this being provided on the outside with a screw. To the under surface of this ring is closely applied a piece of vegetable parchment, and on this is placed a second, but rather thin, brass ring (brass washer), over which we screw a brass cap with a circular aperture 40 mm. in diameter. The exposed brass parts of the dialyser are painted with a suitable varnish. We place the apparatus on small blocks of glass in a crystallising glass, into which we then pour distilled water, while into the dialyser is poured a solution of the substance whose osmotic behaviour is to be investigated. In experiments with extracts of beetroot, solutions of sugar, or salt solutions (*e.g.* solutions of Sodium chloride, Potassium nitrate, etc.), it is easy to determine that the colouring matter, the sugar, and also the mineral substances, can traverse the membrane and pass into the water surrounding the dialyser.

It is now further of special interest, having in view the fact that many substances which can traverse the cell-wall cannot make their way into the protoplasm, to prepare artificially membranes through which substances capable of traversing parchment paper by osmosis cannot pass. We prepare 1 per cent. solutions of Calcium nitrate and Bisodium phosphate. The latter is placed in our parchment paper dialyser, while the former is used as the outside fluid. A precipitation membrane of Calcium phosphate is formed in the vegetable parchment, and if, after a few hours, we add a drop or two of aqueous solution of methyl blue to the Bisodium phosphate in the dialyser, we shall find that the colouring matter does not pass over into the outside fluid. In my experiments, for example, this was still quite uncoloured after twenty-four hours. If we remove the coloured fluid from the dialyser, fit the apparatus with a new piece of parchment paper, replace the outer fluid by water, and pour back into the dialyser

the methyl-blue-containing solution of Bisodium phosphate, we shall find that the colouring matter now soon passes through the parchment into the water. In my experiments the water was clearly tinged at the end of two hours. Precipitation membranes of Calcium phosphate are permeable to Sodium chloride, as we may of course readily ascertain. These experiments, it is to be specially emphasised, are only intended to prove that particular substances which can traverse one membrane are often unable to pass by osmosis through another. Such experiments are obviously of particular interest in examining the behaviour of certain substances towards the cell-wall on the one hand, and the protoplasm on the other. But whether a substance which cannot traverse an artificial membrane is at the same time unable to penetrate into the protoplasm, can only be determined by special observations in each case; and as regards methyl blue, it is to be observed that as a matter of fact it can pass through the protoplasm into the interior of the cells.

If plants of Elodea canadensis are left for twenty-four hours in a 0·0008 per cent. aqueous solution of methyl blue (we use a litre of the fluid), microscopical examination of the leaves shows that their cell-sap is coloured deeply blue. The cells are not dead, for they exhibit protoplasmic movement, and we see therefore that the colouring matter must have traversed the cell-wall and also the protoplasm.[2]

To judge from the results of our investigations, many substances (colouring matters, sugars, vegetable acids, mineral substances) are frequently not able as such to pass by osmosis through the ectoplasm of the protoplasm. But it does not follow that the hyaloplasm is under all conditions impermeable to these substances. Recent investigations of different observers — which, however, are still by no means completed—lead rather to another view. It appears that certain substances which cannot usually traverse the protoplasm, can make their way through it when active accumulation is taking place in the cells. Probably also the hyaloplasm varies in its osmotic properties in consequence of the vital processes themselves, and in accordance with the requirements of the cells, but continued energetic investigation will be required to throw light on these matters.

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[1] Literature: Sachs, *Experimentalphysiologie d. Pflanzen*, 1865, p. 447, where particularly the important work of Nägeli is discussed. Also de Vries, *Archives Néerlandaises*, 1871, T. 6, and Pringsheim's *Jahrbücher*, Bd. 16, p. 588;

Detmer, *Journal f. Landwirthschaft*, 27. Jahrgang, p. 380, as also *Botan. Zeitung*, 1886, No. 30.

[2] See Pfeffer, *Untersuchungen aus d. botan. Institut in Tübingen*, Bd. 2, pp. 223 and 302; and *Abhandlungen der mathem.-phys. Cl. der K. Sächs. Gesellschaft d. Wiss.*, Bd. 16.

59. Turgor and Plasmolysis.

The substances dissolved in the cell-sap (mineral substances, organic acids, sugars, etc.) osmotically attract water into the interior of the cells. As the volume of the cell-sap thus more and more increases, it exerts a pressure on the protoplasm and cell-wall, which, while extensible, are at the same time elastic. The amount of extension in a cell is dependent on the one hand on the magnitude of the osmotic pressure set up inside it, and on the other on the resistance offered by the stretched cell layers (protoplasm and cell-wall).[1]

We can by suitable apparatus directly represent the essential features of turgor. I use for the purpose glass tubes 80 mm. long and 40 mm. wide. We first close one end of such a tube with a piece of pig's bladder, completely fill the tube with an almost concentrated solution of cane-sugar, and then tie a piece of bladder over the other end of the tube also. This so-called artificial cell we now immerse in distilled water. The sugar solution osmotically attracts water, so that the cell-contents, the volume of which more and more increases, exert a constantly increasing pressure on the end membranes. These bulge outwards, but at the same time themselves exert pressure on the cell contents, owing to their elasticity, and thus a considerable tension (turgor-tension) is set up in the apparatus between the sugar solution and the pieces of bladder. When the artificial cell is highly turgescent, it is removed from the water. We pierce the membrane at one end of it with a fine needle, and observe that a stream of fluid at once spurts out from the puncture, while at the same time the membranes slacken. A considerable pressure must therefore be present in the cell when it is in a state of turgescence.

The following experiment, which can easily be made in lecture, is also very instructive:—A small glass is filled with a dilute solution of Potassium ferrocyanide, and a small piece of Copper chloride is dropped into it. The Copper chloride at once surrounds

itself with a precipitation membrane of Cupric ferrocyanide, which becomes stretched since the Copper chloride attracts water from the outside. In this way is formed a turgescent artificial cell (Traube's cell), which, however, rapidly increases in size, and may gradually attain a length of several centimetres. The brown ferrocyanide membrane viz. being stretched, molecules of dissolved Copper chloride pass into it from the inside, and molecules of Potassium ferrocyanide from the outside. These enter into chemical reaction with one another in the membrane, to form the new molecules of Copper ferrocyanide which really effect the growth of the membrane initiated by its extension.[2]

If we mount on a slide in a drop of water hairs from stamens of Tradescantia, or a piece of leaf epidermis from the plant, or a filament of Spirogyra, and run in glycerine or a solution of sugar from the edge of the cover-glass, the appearances mentioned in 58 are exhibited. The cells pass from the turgescent to the plasmolytic condition. Their protoplasm detaches itself from the cell-wall, and draws itself together, while the cell-sap gives up its water to the water-attracting fluids (glycerine, sugar solution) outside. The cells do not by any means at once die as the result of plasmolysis, as is strikingly shown by the fact that the protoplasm of the plasmolysed cells of the staminal hairs of Tradescantia still remains for a long time impermeable to the violet pigment dissolved in their cell-sap.

We must, however, go on to show by experiment that structures made up of many tissues can easily be reduced from a state of turgescence to one of plasmolysis. We employ young flower-stalks of Butomus umbellatus and species of Plantago, leaf-stalks of Tropæolum, etiolated epicotyls of Phaseolus, or main roots of this plant (seedlings germinated in sawdust). Pieces are taken 50–100 mm. in length, and on these, at a distance of 40–90 mm., are made fine ink marks. Pieces of root must first be carefully wiped dry with fine linen. We use best Indian ink, rubbed down in water. To make the marks we use a sable hair brush, which must always be kept perfectly clean. After making the marks, we leave the objects for a few minutes in moist air, to make sure that the ink adheres, and then with a millimetre scale measure the intervals between the marks. The objects are then put into a 10 per cent. aqueous solution of common salt or Potassium nitrate. In these solutions they lose their turgidity, pass into a condition of plasmolysis, become limp, and after a longer or shorter time (four to

twenty-four hours) we can readily determine that the distance between the marks is much less than at the beginning of the experiment. Salt solutions, like glycerine or solutions of sugar, remove water from the cells. The resulting loss of turgidity in the cells results in contraction of the tissues.[3]

When plant structures lose water by withering, they shorten in proportion to the loss of turgidity in their cells. Pea seedlings (developed in sawdust) whose roots have attained a length of about 50 mm., are laid for half an hour in water, in order first of all to make the cells of the root thoroughly turgescent. We now carefully dry the roots with a linen cloth, and place on them two ink-marks, one just behind the tip of the root, the other at a distance of about 25 mm. from the first. If we allow the roots to wither for ten minutes in the air, it will be easy to prove that they have shortened to a not inconsiderable extent. If we now lay the seedlings in water, their roots lengthen again, and the distance between the marks becomes the same as at the commencement of the experiment.[4]

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[1] For a more detailed discussion of Turgor see my *Lehrbuch d. Pflanzenphysiologie*, 1883, p. 213.

[2] See Traube in du Bois-Reymond and Reichert's *Archiv. f. Anat. und Physiol.*, 1867, p. 87.

[3] See H. de Vries, *Untersuchungen über die mechanische Ursache der Zellstreckung*, Halle, 1877.

[4] See Sachs, *Arbeiten des botan. Instituts in Würzburg*, Bd. 1, p. 396.

60. Isotonic Coefficients.

The osmotic energy of a cell is dependent on the quality and quantity of the water-attracting substances present in the cell-sap. In order to make ourselves acquainted with the components of the cell-sap, we first of all select succulent plant structures (*e.g.* leaf-stalks of Heracleum Spondylium, young stems of Rheum, leaves of Crassulaceæ, etc.), and, best after they have been killed by heating in closed vessels on the water-bath, squeeze them in a hand-press. The juice obtained is heated in closed vessels on the water-bath at a temperature of 100° C., so as to coagulate all the proteid matter, and then filtered.* Having evaporated 10 c.c. of the clear juice, and carefully incinerated the residue, we can easily detect the presence of chlorides in an

* The heating of the plant structures and juice is to be carried out in pressure bottles (to be obtained from Desaga, Heidelberg).

aqueous solution of the ash, by adding to it Silver nitrate solution. The presence of glucose is to be determined by means of Fehling's solution. Cane-sugar may be detected by the method given in the Third Section. As a test for Oxalic acid we employ Calcium chloride, and if (first filtering if a precipitate has been produced) we add to the Calcium chloride-containing fluid excess of alcohol, Malic salts separate out if present.

The acid reaction of most plant juices indicates that the bases present are not sufficient to neutralise the whole of the organic acids. For quantitative researches on the composition of plant juices, the student is referred to the directions on p. 570 of the valuable treatise of H. de Vries, cited below.

Often plant juices (*e.g.* that from the leaf-stalk of Heracleum Spondylium) are very rich in glucose, and in such cases this substance is of the utmost importance in determining the osmotic energy of the cell-sap, and therefore also in determining the osmotic pressure of the cells. In other cases, *e.g.* leaves of Solanum tuberosum, the quantity of glucose is far less than that of other bodies.

It is now very important to observe that the osmotic energy of equal quantities of the different substances present in the cell-sap of plants is by no means the same. On the contrary, one substance is able to attract water with great intensity, while another can do so only in a more limited degree. H. de Vries has determined numbers which express the relative attraction for water of one molecule of a body in dilute solution. These numbers he terms the isotonic coefficients of the different substances. As a starting-point for the whole of his researches, H. de Vries selected the force of attraction for water of Potassium nitrate. The isotonic coefficient of one molecule of this compound has been taken as three, so as to render it possible to work with whole numbers.

Putting aside theoretical considerations, we proceed at once to experiments, which will serve to indicate the nature of the reasoning and the method of H. de Vries. We prepare four solutions of Potassium nitrate in water. The first has 0·1, the second 0·12, the third 0·13, the fourth 0·15 molecules of the salt, expressed in grams, per litre (molecular weight of Potassium nitrate, KNO_3, = 101). We further prepare four aqueous solutions of cane-sugar, of which the first contains 0·15, the second 0·2, the third 0·22, and the fourth 0·25 gram molecules of cane-sugar per litre (molecular weight of cane-sugar, $C_{12}H_{22}O_{11}$, = 342). We pour 15 c.c. of

each of these eight solutions into small glass vessels, and in each solution place a small strip, about 1 or 2 mm. long, of the epidermis of the under side of the midrib of the leaf of Tradescantia discolor. These epidermal cells, which I have found to serve excellently well for the experiment, contain in the cell-sap a red pigment. The plant is at our disposal at any time of the year, which is a matter of considerable importance; it is to be grown in the hothouse. We cover the vessels, and leave the strips of epidermis in the fluids for two hours at the usual room temperature, and then examine them under the microscope. What has to be determined is, whether the fluids have induced more or less considerable plasmolysis in the epidermal cells, or whether plasmolysis has not yet set in. The commencement of plasmolysis, with which we are specially concerned, is easily recognised when we use the coloured epidermis of Tradescantia discolor. It is characterised by a just visible retraction of the protoplasm from the membranes of the cells; in what follows we use the term "incipient plasmolysis" with reference to a particular object under examination, when the protoplasm has somewhat contracted in about half the cells of that object.

We shall find that the solution of 0·1 Potassium nitrate, and of 0·15 cane-sugar, do not induce plasmolysis, but those of 0·15 KNO_3 and of 0·25 cane-sugar bring about very considerable plasmolysis. Incipient plasmolysis is observed with strengths of solution between the two, *e.g.* in solution of 0·13 Potassium nitrate, and of 0·22 cane-sugar. Two such solutions would therefore attract water with equal energy; both bring about incipient plasmolysis; their isotonic concentration is the same. The values 0·22, 0·13, stand to one another in the ratio of 1 to 0·591, and if we take the isotonic coefficient of a molecule of Potassium nitrate as 3, that of a molecule of cane-sugar will be 1·77. We see, therefore, that a molecule of Potassium nitrate exerts a greater attraction for water than does a molecule of cane-sugar.

H. de Vries has, by this method, determined the isotonic coefficients of a long series of different substances which occur in the cell-sap. I shall not, however, here proceed further with his important results, but emphatically commend his work to accurate study.[1]

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[1] See H. de Vries, in Pringsheim's *Jahrbücher f. wissenschl. Botanik*, Bd. 14.

61. The Magnitude of the Osmotic Pressure.

To determine the magnitude of the active osmotic pressure in plant structures we may experiment with shoots, employing pieces 1 or 2 mm. in average diameter and 100 mm. long; *e.g.* flower scapes of Plantago, or stems of Lonicera tatarica (which I used), etc., etc. On these we paint ink-marks, at distances of 80 mm. from each other, and then thoroughly plasmolyse them by twenty-four hours' exposure to the action of 10 per cent. solution of common salt. The shortening which is brought about can easily be determined by means of a millimetre scale. The shoots are now stretched by means of the apparatus drawn in Fig. 51. They rest horizontally on a board, *B*, or, better still, on a small sheet of cork. Their thin end is covered with a small block of cork, *K*, which we can firmly fix in position by means of a screw, and round their thicker end is tied a piece of string. The string runs over a pulley, *R*, and supports a scale-pan, *G*, for weights. We load the scale-pan until the distance between the marks on the shoots has become the same as before plasmolysis, viz. 80 mm. That which in the experiment is effected by the weights, is effected in nature by the osmotic pressure. We can thus experimentally determine the magnitude of the pressure within the uninjured plant structure with at least approximate accuracy. If the mean diameter of a cylindrical piece of stem is 1 mm., the corresponding area of its transverse section is 0·785 sq. mm., the area of a circle being given by the formula πr^2 ($\pi = 3{\cdot}141$). If 50 grams are required to stretch to its original length a plasmolysed shoot 1 mm. in diameter, the osmotic pressure within the fresh shoot is equal to about $6\frac{1}{2}$ atmospheres, and, in fact, it frequently attains as high a value as this. In one particular instance, in a stem of Lonicera tatarica, I found it to be 1·4 atmospheres.[1]

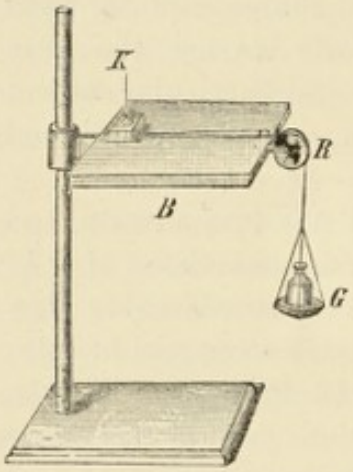

Fig. 51.—Apparatus for determining the amount of osmotic pressure.

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[1] See H. de Vries, *Die mechanischen Ursachen der Zellstreckung*, Halle, 1877, p. 118.

62. The Temperature of Plants.

The temperature of a plant structure is dependent on very many factors. Among these are its organisation, its position in the plant, the quantity of water it contains, its specific heat, its rate of transpiration, and its capacity for absorption, conduction, and radiation of heat. It is therefore clear that, in many cases, it is difficult to account exactly for the particular temperature of a plant structure as experimentally determined. Moreover, many conditions here coming under consideration have not yet been investigated at all or not sufficiently.

Strongly transpiring parts of a plant are often somewhat colder than the air in their neighbourhood, chiefly because a large amount of heat becomes latent in the formation of water vapour. On the other hand, plants which transpire feebly, and are fleshy and succulent in character, often assume a comparatively very high temperature under the influence of direct sunlight. The leaves of a Crassulaceous plant (Sempervivum, Escheveria) which has been exposed to strong sunlight feel quite warm to the touch; their temperature is far higher than that of the more delicate, thinner leaves of plants growing in their immediate neighbourhood. It is very instructive to determine the temperature of succulent plants accurately by means of thermometric measurements.[1] I made observations of this kind on a Cactus (Echinopsis multiplex). A hole is bored right to the middle of the Cactus by means of a cork borer. The hole is cleaned with blotting-paper, and then a thermometer with a cylindrical bulb is introduced into it. After taking care to close the opening tightly, *e.g.* by means of blotting-paper, the plant is placed in the open, in a situation where it will receive during the day direct sunlight. We now observe the temperature of the plant at intervals during the day, and also during the night, and compare the readings obtained with those given by a thermometer hung in the shade. Trustworthy determinations of the temperature of the air are, however, by no means easy to make. It is best to place the thermometer in a large zinc box, hanging in front of a window of a room with a north aspect, at a suitable distance from the ground. The box must be so arranged as to permit circulation of air in it; and further, it must not be placed too near the building. We shall be astonished at the temperature which the Cactus reaches on a warm day in the sunlight. A

particular plant had, at half-past ten in the morning, a temperature of 23° C. By half-past two it had risen to 40·5° C. The temperature of the air at this time, in the shade, was 24·5° C. The same plant, during the afternoon of another day, reached a temperature of 45·5° C. To obtain information as to the temperature within the trunks of trees, we bore to the centre and introduce thermometers into the holes. By slipping short pieces of rubber tubing over the thermometers, the holes can be closed perfectly air-tight. The temperature inside trees is, of course, not the same at points close to the ground as at higher levels, and it is also obviously not a matter of indifference whether or not the plant is exposed during the day to direct sunlight. If we work with moderately thick trunks, *e.g.* 40 cm. in diameter, into which, therefore, the thermometers will penetrate 20 cm., we shall find in general that the temperature is lower than that of the atmosphere during the day, but higher at night, and that the daily maximum of temperature in the tree is attained considerably later than that of the air.

By a simple experiment—which, moreover, is easily performed in lecture—we can demonstrate the fact that dry wood conducts heat more rapidly in a direction parallel to the axis of the trunk than in one at right angles to it. A smoothly planed piece of lime, birch, or oak, is smeared over with a thin layer of wax, by means of a brush dipped into melted wax. We now warm a wire, and press its heated end perpendicularly against the wood. A zone of fusion appears, *S*, Fig. 52, which has the form of an ellipse whose major axis runs parallel with the wood fibres. We can measure the length of the major and minor axes of the ellipse, and so calculate the ratio between the rates of conduction of heat in the wood longitudinally and transversely. If instead of the block of wood we use for the experiment a sheet of glass smeared with a thin layer of wax, the zone fusion which we obtain is not elliptical but circular, because the conductivity of the glass is equal in different directions.

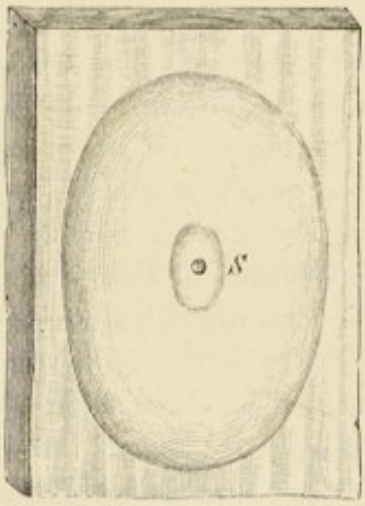

FIG. 52.—Block of wood smeared with wax. *S*, zone of melting.

Since the thermal state of the soil exerts a considerable in-

fluence on vegetation, it is certainly not without interest to make the following experiment:—We procure two cubical zinc boxes, 6 to 8 cm. in diameter. In one we put air-dry soil; the other we fill with an equal quantity of soil which has been more or less completely saturated with water. We now expose the two boxes to direct sunlight for a few hours, preferably in a wooden box and surrounded with a bad conductor of heat (*e.g.* cotton wool), so that the sun-rays will act on the soil almost exclusively from above. If we introduce a thermometer to a depth of 1 cm. into the soil in both boxes, we shall readily make out that the dry soil becomes far warmer than the moist, and I found in observations—conducted, however, in rather a different manner—that a dry peat soil exposed to direct sunlight for an hour and a half had acquired a temperature of 34·3° C. at the surface, while the temperature of the wet soil was only 29·5° C. The lower temperature of the soil soaked with water, as compared with that of the dry soil, is due to the higher specific heat of water, and to the fact that heat is rendered latent by the evaporation of water. Very watery soils we may well term cold—a mode of expression which indeed agrees with the facts. We cannot here investigate further the thermal properties of the soil.[2]

[1] See Askenasy, *Botan. Zeitung*, 1875.

[2] For further information see Detmer, *Lehrbuch d. Bodenkunde*, 1876, p. 256.

63. Differences of Electric Potential in Plants.

To detect differences of electric potential in plant structures we require various delicate instruments, and especially an electrometer (frequently Lippmann's capillary electrometer is used), or a galvanometer. I have satisfied myself that the latter instrument is suitable for the purpose. For a list of firms supplying mirror galvanometers of excellent quality, see the Appendix. The apparatus must of course be set up vibration-free. We further require an electric key and two unpolarisable electrodes. The stands for these electrodes are so arranged that we can easily put the electrodes in any position (see Fig. 53). Each electrode consists of a glass tube a few cm. in length, into the lower end of which prepared clay is kneaded. From the clay project the ends (about $\frac{1}{2}$ cm. long) of well-washed white cotton threads. These threads, which must be washed by means of a vigorous stream of water before the electrodes are used, and in place of

which we may very suitably use small hair pencils, are laid on the objects under examination. The glass tube of the electrodes is filled with a solution of Zinc sulphate; in this dips a zinc rod.* To prevent the pencils from drying, we dip them, when not in use, into water. Renewal of the clay is then only occasionally necessary. The customary kneading of the clay with ½ per cent. alt solution does not appear to be necessary for our experiments; according to my experience spring water is sufficient for the purpose.

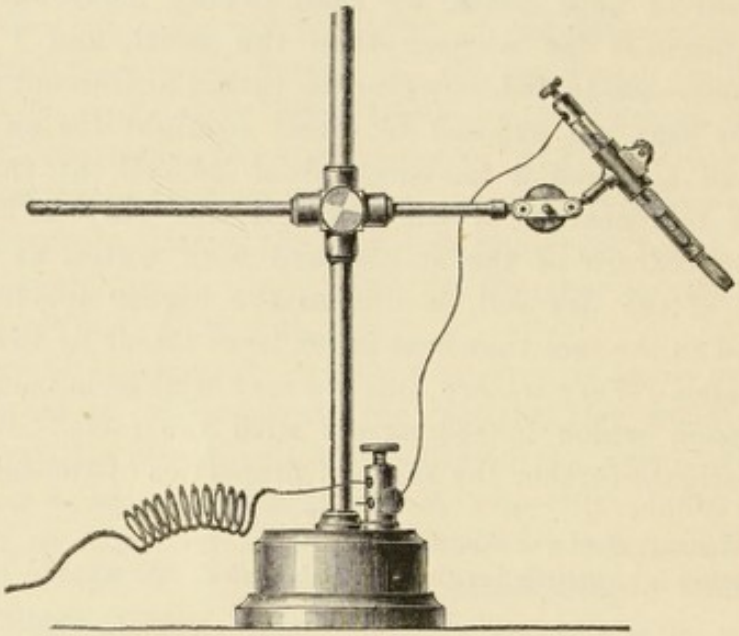

FIG. 53.—Unpolarisable electrodes.

We connect up the electrodes with the galvanometer, one of them directly, the other through a key, by means of which the circuit can be closed or opened as required, while we look through the telescope used for observing the deflection of the galvanometer. We now bring the threads or hair pencils of the electrodes into contact with each other. If no deflection of the needle of the galvanometer takes place when we close the circuit by means of the key, the apparatus is ready for use.

We now proceed to experiments with plants. We lay the electrodes on two points of the surface of a shoot axis not far removed from one another. In many cases, *e.g.* in the case of Aristolochia, no current can be detected when the circuit is closed. It may be just incidentally remarked here that a considerable deflection is

* The zinc rods are, if necessary, to be cleaned by washing with acid, and reamalgamated by dipping in mercury.

naturally observed when one of the electrodes is laid on the periphery of the stem, while the other is placed on an artificially made cross-section.

We further experiment with shoots of Tropæolum, Vitis, Quercus, etc., etc., which dip with their lower ends in water, or with intact seedlings of Pisum and Vicia. In these latter we lay one electrode on the stem and the other on one of the cotyledons. In seedlings considerable differences of potential are observable. In experiments with leaves (we experiment with young leaves, readily wetted, which are left on the plants) one electrode is placed on the midrib of the leaf near its base, the other on the mesophyll near the middle of the leaf. It is found that in almost all cases the leaf-nerves are positive towards the mesophyll, *i.e.* positive electricity streams in the outside part of the circuit from nerve to green tissue.

According to Kunkel, movements of water in the tissues are to be regarded as the chief cause of differences of potential in resting uninjured plant structures. To prove that movements of water can indeed set up differences of potential, we make the following experiment:—A fresh clay cylinder, such as is used for a battery cell, is about half filled with water. We place our electrodes on the outside of the cell, one near the base, the other at a point above the level of the water. A current then flows through the galvanometer from the upper to the lower electrode.

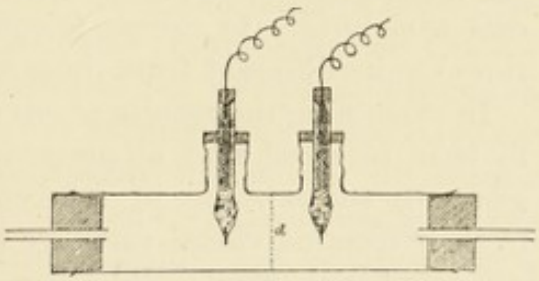

FIG. 54.—Apparatus for investigating electric potential in plant structures. Represented in section. (After Haake.)

While differences of potential in plant structures may be due to movements of water, etc., they are chiefly due, as Haake proved, to quite different processes, viz. metabolism and respiration, which are intimately associated with the vitality of the protoplasm.

To prove this interesting fact we require the apparatus indicated in section in Fig. 54, which was employed by Haake. It consists of a glass tube, 20 cm. long and $3\frac{1}{2}$ cm. internal diameter, on one side of which are fused two glass tubes, 2 cm. in diameter and 1 cm. high, and $2\frac{1}{2}$ cm. apart. Over these are slipped pieces of thin rubber tubing, 5 cm. long, which, as is clear from the figure, fit air-tight round the electrodes which pass through them.

The projecting ends of the electrodes are fixed to the stands. The two ends of the main glass tube are fitted with rubber stoppers, through which pass glass tubes. If now we place in the moist chamber a pea seedling 10–15 cm. long, and lead through the apparatus a slow current of air, saturated with aqueous vapour by passage through a small U-tube filled with wet glass wool, we get, according to Haake, a very large deflection of the galvanometer when one electrode is placed on the neck of the root, the other on the stem of the seedling. The deflection becomes however slight if now, for about a quarter of an hour, we pass a slow stream of Hydrogen through the apparatus. The Hydrogen is purified by passage through a solution of Potassium permanganate, and saturated with aqueous vapour by being led over moist glass wool. Renewed access of air again leads to considerable deflection of the galvanometer. In these experiments care must always be taken to keep the plants in the dark, so as to prevent assimilation.

If, therefore, we deprive plant structures of Oxygen, we bring about a change in their electric condition, and this justifies us in concluding that metabolism and respiration must be regarded as a cause of the differences of potential to be observed in plants. It is true, killed plants (*e.g.* pea seedlings killed by steam) also exhibit differences of potential, but these do not undergo any marked change on withdrawal of Oxygen, which proves that they owe their origin to causes (chemical changes in the dead structures) quite different from those taking place in the living plant.

In examining the results of our observations, it is very important to bear in mind that at present we are only able to demonstrate generally the existence of differences of potential in plants. As to their true individual value we know nothing. The cells may be compared with small voltaic elements, but the galvanometer indicates only the difference between the strengths of the currents leaving the different cell complexes brought into contact with the electrodes. Even if the galvanometer no longer indicates any current at all, electric actions might still be going on; the currents sent through the galvanometer in opposite directions from the two points might be equal.

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[1] See Haake, *Flora*, 1892, and Kunkel, *Arbeiten d. botan. Instituts in Würzburg*, Bd. 2. In Kunkel, see also observations on differences of potential in resting and mechanically stimulated leaves of Mimosa, and on the diminution of current (negative variation) exhibited on stimulation.

IV. THE MOVEMENT OF GASES IN PLANTS.

64. Concerning the Behaviour of Gases in General.

A test-tube is filled with plant juice, *e.g.* beetroot juice, obtained by rubbing pieces of peeled beetroot on a grater, squeezing the pulp in a cloth and finally clearing by filtration. We also fill a small dish with the beetroot juice, invert the test-tube in it, and then replace the juice in the test-tube by Carbon dioxide gas. If we let the apparatus stand for some time, we shall observe that the juice rises in the test-tube. The Carbon dioxide is absorbed by the fluid, and in the same way the sap present in the intact cell is able to absorb Carbon dioxide gas brought into contact with it. Oxygen and Nitrogen are absorbed by aqueous solutions much less energetically than Carbon dioxide gas.

Air-dry plant structures, *e.g.* seeds, can also absorb not inconsiderable quantities of Carbon dioxide. In a glass tube fused up at one end, we place 15 to 20 air-dry seeds of Phaseolus multiflorus, and then push in a small piece of cork or some glass wool, so as to confine the seeds to the closed end of the tube. The tube is now placed with its closed end downwards, a rapid current of Carbon dioxide is led into it, and it is then closed with the thumb and quickly inverted with its mouth under mercury. The mercury gradually rises in the tube, the seeds in the course of several days absorbing some cubic centimetres of Carbon dioxide, and if we accurately determine the volume of the gas at the beginning and at the end of the experiment respectively (see method in 13), we can estimate exactly the amount of Carbon dioxide fixed by the seeds.[1] According to Borodin, soaked bean seeds absorb not much more Carbon dioxide than air-dry seeds, a statement which challenges further investigation.

As shown by Graham and Bunsen, the rate at which gases traverse in opposite directions porous septa which exert no specific force or attraction on these gases, varies inversely as the square root of the specific gravity of the gases. Indeed, it may easily be shown that Hydrogen, *e.g.*, passes much more rapidly through porous partitions than does atmospheric air. In my experiments I used a glass tube 15 mm. in diameter and about 40 cm. long, closed at one end with a dry plate of clay 5 mm. in thickness. The clay plate may be easily fixed on the end of the tube with sealing-wax. If the glass tube is now filled with

Hydrogen, and then rapidly inverted under water, the fluid quickly ascends to a considerable height in the apparatus, since in a given time a larger quantity of Hydrogen passes through the clay into the atmosphere than atmospheric air into the tube.

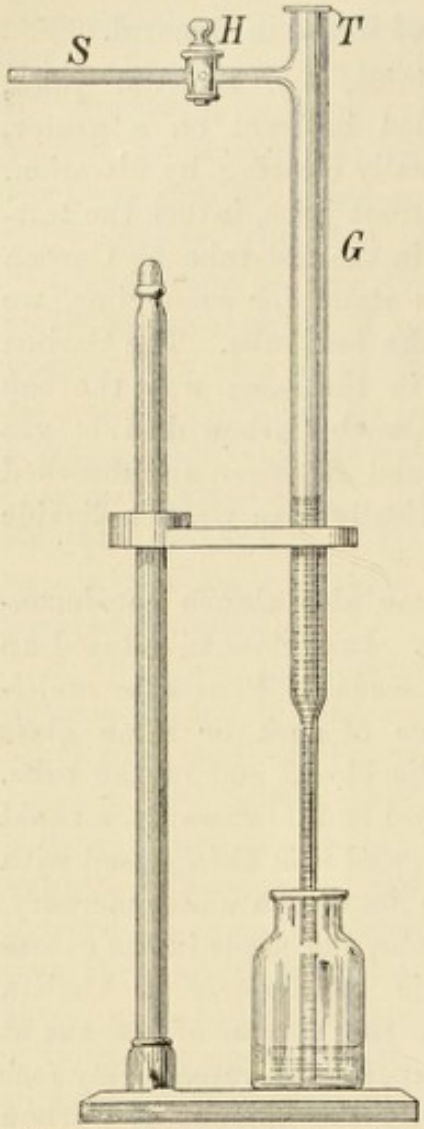

FIG 55.—Apparatus for investigating the diffusion of gases.

It is more convenient to work with the apparatus represented in Fig. 55, which I also used. The disc of clay *T* closed the upper end of the glass tube *G*, which at the bottom dips into water. The stopcock *H* being open, Hydrogen is led into the apparatus through *S*. On closing the stopcock again, the water gradually rises in the tube *G*. This arrangement may also be employed for many of the experiments which follow.

Carbon dioxide having a high specific gravity traverses porous septa, such as plates of clay, at all events much more slowly than atmospheric air; but the very opposite is the case, if we separate the Carbon dioxide from the atmospheric air by a septum composed of a substance which exerts a specific attraction (gas absorption) on the Carbon dioxide. I closed a glass tube with a thin rubber membrane (fastened on with a piece of elastic), filled the tube with Carbon dioxide, and inverted it under mercury. The mercury gradually rose higher and higher in the tube. It is true the mercury does not rise rapidly, and it is hence necessary to keep the experiment going for a good time (say twenty-four hours). If it is not desired to make accurate quantitative experiments, but merely experiments for demonstration purposes, it is well to have, in addition to the tube closed with the rubber membrane, another of the same dimensions, but fused up at its upper end. The position of the mercury in this second tube indicates how far the

volume of the gases is effected during the experiment by conditions of temperature and atmospheric pressure. It is now seen after some time that the mercury in the tube closed with the membrane stands considerably higher than the other, and this is due to the fact that owing to the absorption of the Carbon dioxide by the rubber, the gas, in spite of its high specific gravity, passes more rapidly through the membrane than can the atmospheric air.

I closed a glass tube at one end with a fresh piece of Nerium Oleander leaf, filled the tube with Carbon dioxide, and inverted it under mercury. The mercury rose fairly high in the tube. In this experiment the best way to make the tube air-tight is to slip over it a piece of cork with a hole in the middle, till the top of the tube comes exactly flush with the surface of the cork, and fix this in position, so as to serve as a rim, by means of sealing-wax applied below. The cork rim is then smeared on the top with a mixture of 1 part of yellow wax, 1 part of olive oil, and 1 part of melted mutton suet. The piece of leaf is placed on it with its upper surface, which is free from stomata, downwards, and the operation is completed by smearing the edges of the leaf with the cement.

More accurate results are obtained by the following method of investigation. A perforated piece of cork or elder pith is pushed over the well-ground end of a glass tube 5–6 mm. wide, and 50–100 cm. long, so as to form a rim. We warm well and smear it with melted sealing-wax of the finest quality. We now lay the object to be investigated (*e.g.* a dry, perfect piece of Hedera Helix leaf, the upper side of which, as is known, is free from stomata, or a thin lamella of cork, cut, it may be, with the microtome) on a piece of cork, invert the glass tube, and bring it with moderate pressure in contact with the tissue while the sealing-wax is still melted. As a rule, we get in this way a perfectly air-tight closure. After cooling thoroughly, the tube is laid nearly horizontal, carefully filled entirely or only in part with mercury, and then fixed vertically with its open end dipping under mercury. We now displace part of the mercury in the tube by dry Carbon dioxide, and let the apparatus stand in a place looking to the north. The position of the mercury in the tube is read, with the necessary precautions, the temperature and barometric pressure being simultaneously noted; and if we repeat this frequently, *e.g.* once every day, we obtain the following result. In the apparatus

closed with a lamella of cork, the mercury rises very slowly, *e.g.* only 1 mm. per day. No rise at all takes place if we experiment with dry pieces of Hedera Helix leaf. A fairly rapid rise of the mercury is exhibited, if we lay on the pieces of tissue, after they have been fixed, one end of a strip of blotting-paper the other end of which dips into water, so that they imbibe water. It is found, therefore, that gas dialysis, in which, of course, gas absorption plays an important part, can only take place vigorously when the membranes of the plant tissues are imbibed with water. Dry membranes do not allow gases to dialyse, or they permit them only very slow passage (periderm). Carbon dioxide traverses plant membranes by dialysis more rapidly than Nitrogen and Oxygen.[2]

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[1] See Borodin, *Mémoires de l'acad. imp. de St. Pétersbourg*, T. 28, No. 4.

[2] For the literature see Detmer, *Lehrbuch d. Pflanzenphysiologie*, 1883, p. 97, and Pfeffer, *Handbuch d. Pflanzenphysiologie*, Bd. 1, p. 86. Experimental researches which are of interest have been carried out by N. J. C. Müller (see Pringsheim's *Jahrbücher*, Bd. 7). Also see Wiesner, *Sitzungsber. d. Akad. d. Wiss. zu Wien*, 1889, Bd. 98, Pt. 1, p. 693.

65. The Intercellular System of Plants.

The intercellular spaces of plants, which are so important in connection with gas exchange, ventilation, and the hereafter-to-be-considered formation of aqueous vapour in the plant, are found particularly between the cells of the parenchyma, but also in other places. They originate either schizogenously or lysigenously, and in the latter case are often of considerable diameter. In the parenchyma the intercellular spaces usually occur between the rounded angles of the cells as triangular intercommunicating canals. We first submit to microscopic examination delicate transverse sections from the cotyledons of seeds of Lupinus luteus, cut after soaking the seeds somewhat. We soon see the rather narrow intercellular spaces between the cells. The air spaces are naturally of special importance for normal germination, which is associated with active gas exchange. The intercellular spaces are also readily made out between the cells of the cotyledons of Lupinus, when they have risen above ground and become green.

We further prepare transverse sections through an internode of Zea Mais (see Fig. 56). Air-dry material is quite suitable for our purpose. The vascular bundles are not arranged in a circle,

but are distributed through the whole ground tissue. The cells of this last are very large, and our attention is at once arrested by the somewhat large intercellular spaces which appear as triangular lacunæ between the cells. Each of the collateral vascular bundles is surrounded by a sheath of thick-walled sclerenchyma

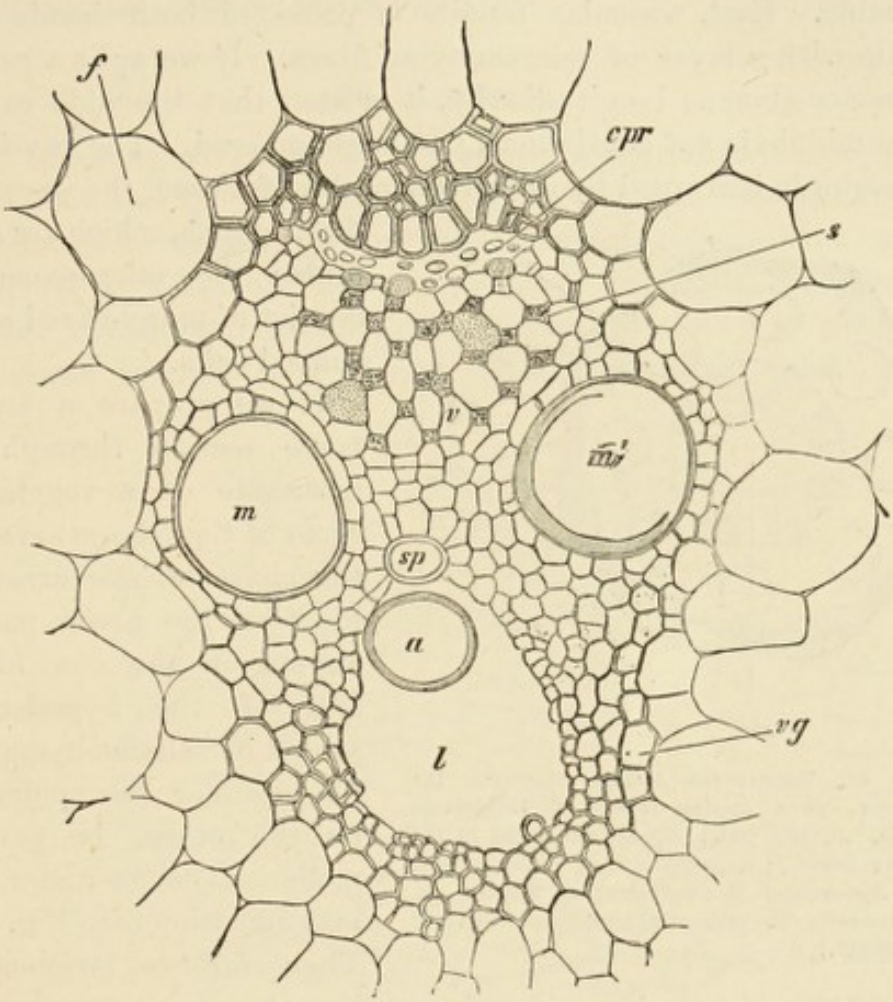

FIG. 56.—Transverse section through a vascular bundle from the inner part of an internode of the stem of Zea Mais. *a*, segment of an annular vessel; *sp*, spiral vessel; *m* and *m'*, vessels with simple pits; *v*, sieve tube; *s*, companion cell; *cpr*, crushed primary phloem; *l*, intercellular passage; *vg*, sheath; *f*, cell of the ground tissue. Magn. 180. (After Strasburger.)

cells closely packed without intercellular spaces. In the phloem of the vascular bundles the sieve tubes are visible; while in the xylem several large vessels at once strike the eye. On the inner side of the vascular bundle we observe a wide intercellular space. This is of lysigenetic origin, while the intercellular spaces of the ground tissue are developed schizogenetically.

If transverse sections of the stem of Juncus glaucus are

examined under the microscope, it is seen that, under the strongly cuticularised epidermis, green tissue and groups of sclerenchymatous fibres alternate with each other. Under the bundles of sclerenchyma fibres are to be seen large cavities filled with air; and we further perceive embedded in the ground tissue many vascular bundles, the wood and bast of which are readily distinguishable. Each vascular bundle is provided both inside and outside with a layer of sclerenchyma fibres. If we split a haulm of Juncus glaucus longitudinally, it is seen that the wide cavity in the middle is not continuous, but is chambered. The cavity of the haulm is traversed by numerous plates of tissue, the so-called diaphragms, which are seen under the microscope to consist of many-rayed star-shaped cells.

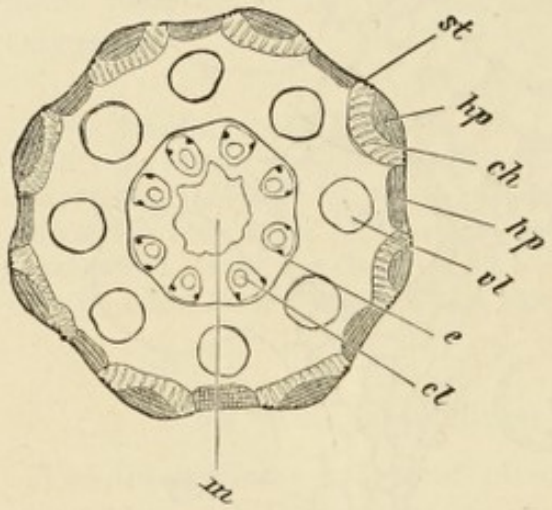

FIG. 57.—Transverse section through the internode of a sterile shoot of Equisetum arvense. *m*, the pith; *cl*, carinal canal in the vascular bundle; *e*, endodermis; *vl*, vallecular canal; *hp*, strand of sclerenchyma fibres; *ch*, green tissue; *st*, stomatal apparatus. (After Strasburger.)

If we prepare a transverse section through an internode of a vegetative shoot of Equisetum arvense, the characteristic arrangement of the green parenchyma on the one hand, and of the hypodermal strand of sclerenchymatous fibres under the epidermis on the other, is readily made out even under low magnification (see Fig. 57). Then follows large-celled cortical tissue enclosing wide air cavities, the so-called vallecular canals. The circle of vascular bundles is surrounded by an endodermis, and each bundle is clearly differentiated into wood and bast. In the wood a wide intercellular passage, the carinal canal, is easily seen. Finally, the hollow pith and the comparatively narrow intercellular spaces between the cells of the cortex and pith are to be noted.

On microscopic examination of a delicate transverse section from the leaf-stalk of Nymphæa alba we perceive an epidermis free from stomata, and below this a ring of collenchyma. In the peripheral region of the leaf-stalk occur the vascular bundles, arranged in a circle; but there are also fibro-vascular bundles

distributed in the ground tissue. The ground tissue is further traversed by numerous more or less wide air canals. Into these last project stellate internal hairs, each of which springs from a single cell of the ground tissue limiting the canal.[1]

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[1] Literature: de Bary, *Comparative Anatomy of the Phanerogams and Ferns*, and Strasburger, *Practical Botany* (Hillhouse).

66. Lenticels.

To acquaint ourselves with the very widely distributed structures known as Lenticels, we investigate twigs of Sambucus nigra. In transverse sections which have been prepared from young branches, we make out that, immediately below the epidermis, is present hypodermal collenchyma, interrupted only at places by the green cortical parenchyma, which, at such points, itself

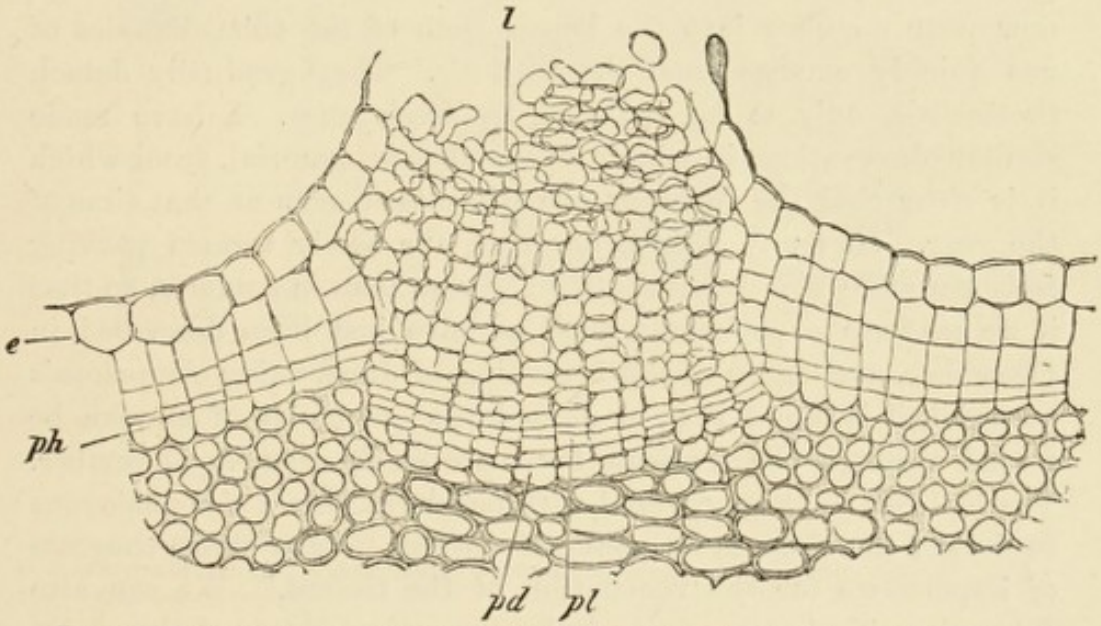

FIG. 58.—Transverse section through a lenticel of Sambucus nigra. *e*, epidermis; *ph*, phelloderm or cork cambium; *l*, packing cells; *pl*, cambium of the lenticel; *pd*, phelloderm. Magn. 90. (After Strasburger.)

reaches up to the epidermis. The inner cortical tissue consists entirely of green cells, and surrounds the circle of vascular bundles. Examination of older branches of Sambucus shows that important changes have taken place. Close under the epidermis a cork tissue has developed, the yellowish cells of which shut in the living tissue of the cortex. But this enclosure is not complete, since the twigs are provided with numerous lenticels. These may be detected even with the naked eye as brownish spots,

and on microscopical examination of transverse sections we find (see Fig. 58) that where lenticels occur the epidermis is burst open, and that the lenticels are themselves filled with a powdery mass of dark-brown coloured cells (packing cells). These packing cells are, as is known, produced, as also are the cells of the phelloderm, by the cambium of the lenticels. The packing cells, which as rapidly as they undergo disorganisation from without are replaced by the cambium, are so arranged that spaces are left between them filled with air, and these communicate with the intercellular spaces of the internal tissue of the twigs. To prove this fact experimentally, we fasten a shoot of Sambucus nigra, Salix, or Pavia rubra, covered with periderm and lenticels, in the shorter limb of a bent glass tube by means of a melted mixture of 2 parts wax and 1 part Colophonium. The cut surface at the top of the branch is also well sealed with the mixture, and the preparation is placed in a glass cylinder filled with water. If we now pour mercury into the longer limb of the tube, bubbles of gas quickly emerge from the lenticels. They gradually detach themselves, only to be replaced by fresh ones. I have made similar observations in winter on Sambucus material, from which it is clear that the lenticels are not closed even at that time of the year. In many plants, however, the newly formed packing cells are more closely arranged in winter than in summer, so that if we perform experiments, such as have just been described, in December, and again at the beginning of June, using Ampelopsis material, it is found that in June large quantities of air can be forced through the lenticels far more easily than in December. The lenticels in twigs covered with periderm play a rôle analogous to that of the stomata on young structures. Like these, they are of importance for the ventilation of the tissues.[1] We can also determine this fact very simply by cementing shoots of the plants at both ends with wax mixture, and then putting them in warm water. The warmed air now escapes in bubbles from the lenticels.

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[1] Literature: Stahl, *Botan. Zeitung*, 1873, and Klebahn, *Jenaische Zeitschrift für Medicin und Naturwissenschaft*, Neue Folge, Bd. 10.

67. Stomata and their Importance in the Gas Exchange.

A very favourable object for the study of stomata is the leaf of Iris florentina. On microscopic examination of delicate transverse sections, it is seen (see Fig. 59) that the guard cells of the stoma-

tal apparatus contain chlorophyll grains. The slit of the stoma, and the respiratory cavity below the slit, are also easily made out. The depression above the stoma is caused by the encroachment of the adjoining chlorophyll-free epidermal cells on the guard cells, which they partly cover.

This depression of the stomatal apparatus below the surface is not found in leaves of Tradescantia virginica, as is shown by examination of thin transverse sections. It is characteristic of

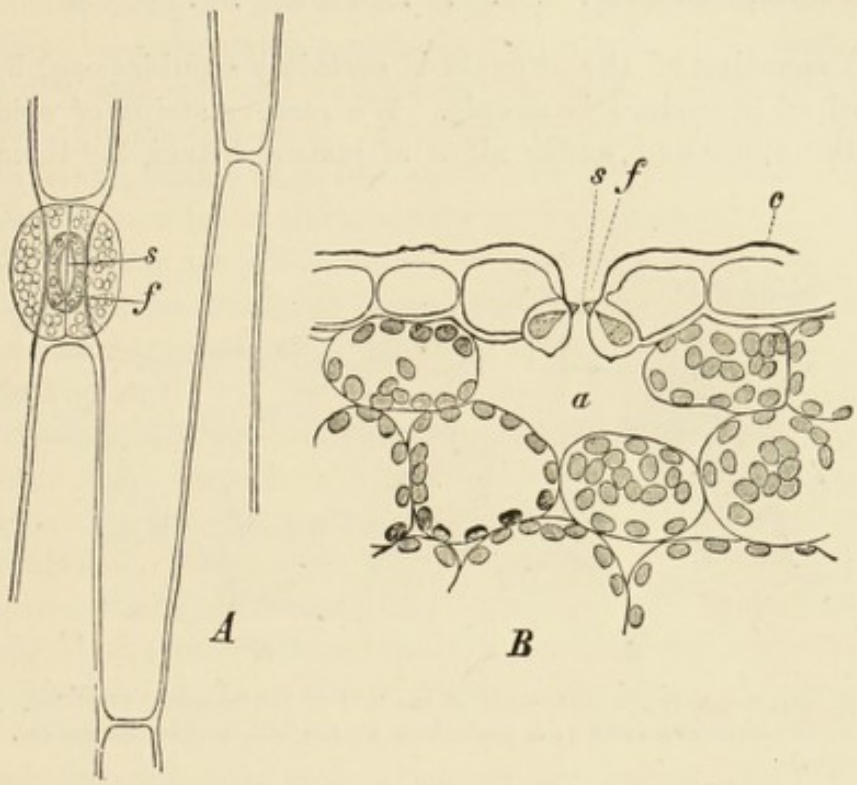

FIG. 59.—Epidermis from the under side of the leaf of Iris florentina. *A*, from above; *B*, in transverse section; *f*, depression; *s*, stoma; *c*, cuticle; *a*, respiratory cavity. Magn. 240. (After Strasburger.)

the stomata of Tradescantia virginica that they are almost invariably surrounded by four epidermal cells, which, as can easily be seen, contain beautiful nuclei. To prove this we examine a shred of epidermis stripped from the under side of a Tradescantia leaf. The stomata are far less numerous on the upper side of the leaf than on the under side (see Fig. 60). Good material for the study of the stomatal apparatus is also afforded by leaves of Hyacinthus orientalis and Lilium candidum.

In studying stomata, it will soon be observed that the number of them on equal areas of the leaf is very different in different

plants, or on the upper and lower surfaces of the same leaf. So, *e.g.*, according to Weiss,[1] the number of stomata on 1 sq. mm. of leaf surface is in :—

	Upper side.	Under side.
Acer platanoides	0	550
Brassica oleracea	219	301
Helianthus annuus	175	325
Ficus elastica	0	145
Orchis latifolia	20	67
Nymphæa alba	460	0

The counting of the stomata is certainly troublesome, but the method of procedure is simple. We remove strips of epidermis from the upper and under sides of mature leaves, lay them in a

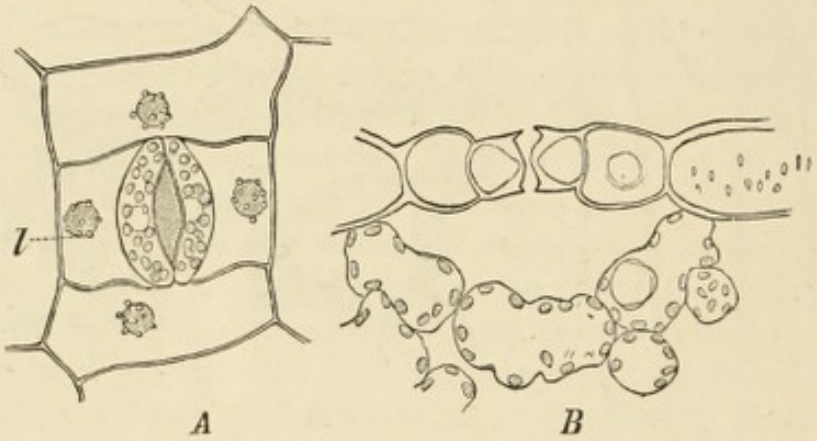

FIG. 60.—Epidermis of the under side of the leaf of Tradescantia virginica. *A*, from above; *B*, in transverse section; *l*, leucoplasts on the cell nuclei. Magn. 240. (After Strasburger.)

drop of water on the slide, and put on the cover-glass. We then count the stomata observable in the field of view, and take the mean of a series of such observations. The actual area of the field of view is easily calculated from its diameter as determined by means of a stage micrometer. It only remains to estimate from the number of stomata on this area, the number on unit area, *e.g.* 1 sq. mm.

In speaking of the stomata of plants, it is important to make mention of a peculiarity which is of great significance in connection with the gas exchange, and also in connection with the process of transpiration to be discussed later. It is that the slit between the guard cells of the stoma is by no means always of the same

width. We can easily prove that the slit of a stoma under certain conditions is more or less widely open, while under other conditions it is closed. These remarkable changes are due to variations in the turgidity of the guard cells and of the adjoining epidermal cells of the stomatal apparatus, and, neglecting complicated details, we will make some experiments demonstrating the main facts.[2]

We examine first a tangential section of a leaf of Amaryllis formosissima, cut off in the daytime, and find the stomata open. If, on the other hand, we use a half-withered leaf of the same plant, we observe that the stomata are closed. It is well to examine the section dry, and only add water after we have satisfied ourselves that the stomata are shut. Addition of water now causes the stomata in the course of a few minutes to open widely, owing to considerable increase in the turgidity of the guard cells. If, however, the sections are left in water for some time, the stomata close again, because the turgidity of the epidermal cells also gradually increases largely, so that the pressure of the guard cells is little by little overcome.

If, however, sections are prepared from Amaryllis leaves which have been well sunned, it is impossible to cause the stomata to close by treatment with water, since the guard cells, in consequence of assimilation, contain such large quantities of osmotically active substances, that they turgesce energetically enough to hold the turgidity of the neighbouring epidermal cells in equilibrium.

The stomata of other plants behave like those of Amaryllis. On the other hand, we observe a difference of behaviour in plants in which the epidermal cells adjoining the guard cells are of no great importance in connection with the stomatal apparatus. If strips of epidermis from Orchis leaves (I used Orchis mascula) are examined first of all in a drop of water, it is seen that the stomata never close, but remain open. If, on the other hand, the section be laid on the slide in a drop of cane-sugar solution, the stomata close fairly quickly. The stomata of Lilium candidum behave in a similar manner. Copious supply of water to the plant, to leaves removed from the plant, or to the sections, opens the stomata. Withering of the leaves, or withdrawal of water from the sections, reduces the turgidity of the guard cells, and causes the stomata to close.

The stomatal apparatus of many plants reacts also to changes in illumination. The stomata of Amaryllis formosissima, for

example, are closed at night. Direct sunlight causes them to open widely, and if we suddenly cut off the light from leaves previously brightly illuminated, we shall, after a few hours, find the stomata closed. We may also, as I have often done, experiment with leaves of Amaryllis removed from the plant, and placed with the cut end in water. If we examine sections of such leaves after they have been brightly illuminated for several hours, we always find the stomata open, whereas leaves which have been kept in the dark exhibit closed stomata. Schaefer (l.c. p. 194) showed directly that the closing in darkness is due to diminished turgidity of the guard cells, and not to increased turgidity of the neighbouring epidermal cells. In investigating the influence of conditions of illumination on the stomatal apparatus of Orchis mascula, I could not determine any marked difference as regards width of slit between leaves exposed to light and leaves kept dark. Leitgeb also has recently obtained similar results with Orchis (see his cited work, p. 160).

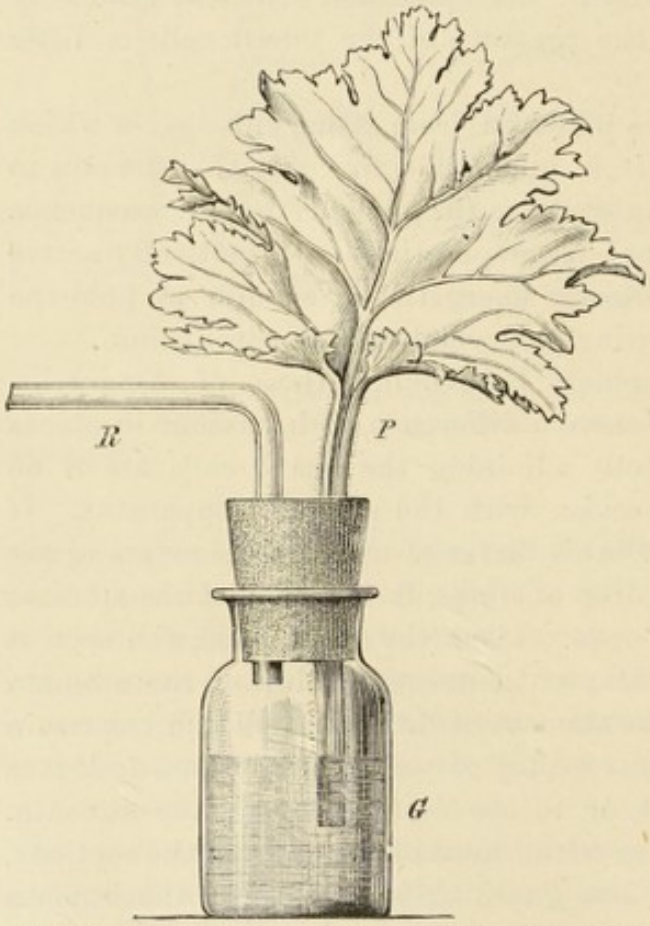

FIG. 61.—Apparatus for proving that the air can pass through stomata.

The influence of induction currents on the stomatal apparatus is very surprising. We take a strip of epidermis from the under side of a leaf whose stomata can open widely. I obtained the best results with leaves of Orchis mascula, gathered in the daytime and kept for some time in water. The strip of epidermis was placed on a slide like that described in 54, and depicted in Fig. 45, which is designed for studying the influence of electricity on plant-cells. If we focus a few stomata, and then pass induction currents through the section,

the stomata in a short time close under the eye of the observer.*

It is very instructive to determine experimentally that the stomata are open and form free outlets from the intercellular spaces. We may first make the following experiments, using an uninjured leaf of Primula sinensis. The apparatus depicted in Fig. 61 is employed. The glass *G*, fitted with a two-holed rubber stopper, is half full of water. One limb of the tube *R*, which is bent at right angles, passes just through the stopper. The leaf-stalk *P* dips into the water. If we rarefy the air in the apparatus by sucking with the mouth, fresh air passes into the stomata of the leaf and emerges in a stream of bubbles at the cut surface of the leaf-stalk situated below the surface of the water. If we experiment with other leaves, we shall very often find that the reduction of pressure effected by mere mouth suction is not sufficient to cause an escape of air from the cut surface of the leaf-stalk. We must then connect the end of the tube *R* with an air-pump in order to get better exhaustion.

We can, however, show conversely that air forced into the leaf-stalk passes into the intercellular spaces, and escapes from the stomata. I also used leaves of Primula sinensis for experiments of this kind. If we dip the blade of the leaf under water, and then, taking the leaf-stalk into the mouth, blow vigorously into it, bubbles of various sizes will be seen to detach themselves from the leaf. There may not be, as we might perhaps expect, a fine stream of bubbles from each stoma, but the air forced out collects to form larger bubbles, which then here and there rise from the surface of the leaf. If the blade of the leaf is left for some time under water, it becomes impossible to drive air through it by mere blowing. The stomata, in fact, gradually become blocked up with water retained by capillarity, and this state of affairs may be very rapidly brought about in the leaf of Primula sinensis by applying the lips to the cut end of the leaf-stalk, and exerting suction while the blade is under water. The stomata and the intercellular spaces are thus injected with water, and the blade consequently changes in colour, becoming also translucent. We are now unable to drive air through the leaf by vigorous blowing.

* This closure of the stomata is due to the death of the guard cells under the influence of the current. The stomata, therefore, do not subsequently open again, and the contents of the guard cells often rapidly break up.

To demonstrate clearly the fact, which is of great importance in explaining the above experiments, that water retained by capillarity can hold itself in equilibrium against not inconsiderable pressures, we make the following experiment, using the apparatus depicted in Fig. 62. The shorter limb of a bent glass tube, a few millimetres in diameter, is drawn out at its end to a fine capillary. We now place the tube in a glass cylinder filled with water, with the capillary opening a few centimetres below the surface of the water, and pour mercury into the longer limb of the tube till a pressure of about 20 cm. of mercury is attained. We observe that a fine stream of bubbles escapes from the capillary end of the tube. The mercury sinks further and further in the long arm of the tube till its pressure, as indicated by the difference in level in the two limbs, is reduced to a few centimetres, and then no further depression of the mercury takes place, and at the same time the stream of air ceases. The fine open end is blocked with water. The final mercurial pressure is held in equilibrium by capillary attraction of water into the drawn-out portion of the tube.

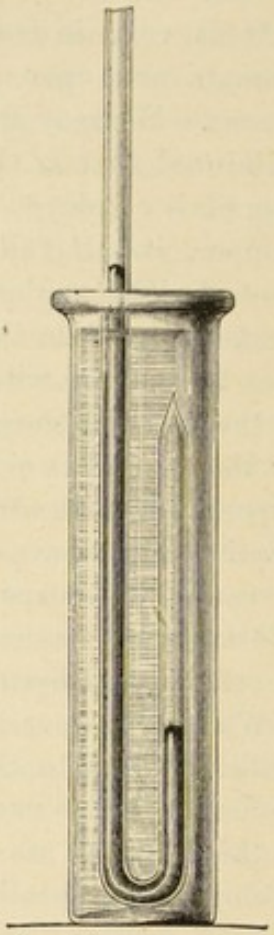

Fig. 62.—Apparatus for investigating the permeability of capillaries to air.

If we immerse in water the blade of a leaf of Caltha palustris, or Nymphæa, or part of a leaf of Allium Cepa, and blow into the leaf-stalk, or, in the case of Allium, into the open end of the leaf, we succeed here again, in this simple manner, in forcing air through. The surface of the immersed portion of the Allium leaf exhibits a beautiful silvery lustre, due to its being covered with a layer of air, which causes total reflection of light. If by rubbing with the finger we remove the adherent layer of air from any parts of the leaf, the water obtains access to them, and they take on a green colour. Air now escapes from the leaf on blowing, only where the epidermis is still provided with its silvery covering. No air bubbles are set free from the wetted parts since the stomata are here stopped up by water attracted by capillarity, and

the comparatively small pressure produced by mere blowing with the mouth is insufficient to force the water out of these stomata.

In experiments respecting the passage of air through the stomata, it is often suitable to adopt the following method of procedure. We fix air-tight in the shorter limb of a bent glass tube, a leaf-stalk bearing an uninjured lamina, or a stem bearing leaves (I used, *e.g.*, the end of a Camellia shoot bearing a terminal bud and a single leaf). The closure is made air-tight in different ways according to circumstances. Frequently it is sufficient to seal up with a mixture prepared by melting together equal parts of yellow wax, olive oil, and melted mutton suet. In other cases we use a piece of rubber tubing; or we first fit the tube with a perforated cork, and pass the leaf-stalk or shoot through this, and then complete the operation by smearing carefully with wax mixture. If we pour mercury into the longer limb of the bent glass tube, and place the apparatus in a cylinder filled with water, the compressed air escapes from the stomata, and bubbles of air of various sizes pass from the leaf-blade and rise through the water.[3]

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[1] See Weiss, in Pringsheim's *Jahrbücher*, Bd. 4.

[2] Literature: Mohl, *Botan. Zeitung*, 1856; Schwendener, *Monatsberichte d. Berliner Akademie d. Wiss.*, 1881; Leitgeb, *Mittheilungen d. botan. Instituts zu Graz*, Bd. 1, Jena, 1886; Schaefer, *Jahrbücher f. wissensch. Botanik*, Bd. 19.

[3] With reference to what is here said, see especially Sachs, *Handbuch der Experimentalphysiologie d. Pflanzen*, 1865, p. 252.

68. Positive and Negative Gaseous Pressure in Plants.

The air in the intercellular spaces of submerged plants, in which, as is well known, stomata are usually absent, is often at a positive pressure. This excess of pressure may be brought about in various ways, but the assimilatory activity of the green parts of the plants, under the influence of sunlight, is of special importance. If we place shoots of Elodea in spring water, and expose them to direct sunlight, a stream of bubbles will spring from the cut end of the stem, as we have proved in 11. This evolution of Oxygen ceases almost at once if we prevent access of light. If uninjured plants of Elodea or Ceratophyllum, or shoots of these plants (I experimented with shoots of Elodea) whose cut surfaces have been smeared with wax, are exposed under water to the influence of direct sunlight, there is no escape of bubbles of gas. The Oxygen

produced collects in the intercellular spaces; it does not pass out into the water by dialysis at the same rate as it is formed, and consequently the gas in the intercellular spaces soon stands at a positive pressure. In fact, if we prick the stem of one of the plants with a needle, a very rapid, but it is true instantly diminishing, stream of bubbles at once springs from the wound. If we repeat this experiment with plants which have been kept in darkness for a time under water, the injury does not lead to this copious liberation of gas, since the Carbon dioxide formed in the process of respiration cannot much affect the pressure of the intercellular gases. It is easily soluble in water, and can readily pass over by dialysis from the plants into the medium surrounding them. To acquaint ourselves more minutely with the intercellular system of Elodea canadensis, we subject transverse sections of the stem to microscopic examination. Under the feebly differentiated epidermis lies a comparatively well-developed cortex, which in turn surrounds the axile bundle. The cells of the cortical tissue have small intercellular spaces between them, but besides these there is present in the cortex a circle of large air-canals.

The air in the intercellular spaces may, however, under certain conditions have a slight negative pressure, being more rarefied than the atmospheric air. This may be due to a variety of conditions, but I will consider only one case. If branches of trees are exposed to direct sunlight, their cortical tissue especially will frequently attain a higher temperature than the surrounding air. The gases in the intercellular spaces expand, and if lenticels are present some of it escapes through them. The air remaining in the intercellular spaces is now more rarefied than that of the atmosphere.

The fact that the gases in the elements of the wood, especially at the time of active transpiration, stand at a considerable negative pressure is very important, and is of great significance in the explanation of many phenomena of plant life. We shall later return to this remarkable phenomenon of the negative pressure of the air in the wood, but will first fix our attention on some conditions which stand in relation to it.

It is first to be noticed that the cork tissue, if we leave out of consideration lenticels, does not allow gas to pass through it, even under considerable pressure.[1] By means of sealing-wax I fixed a thin transverse section of cork (which had been prepared from a small cork by means of a razor) air-tight over the opening of the

shorter limb of a bent glass tube, a few millimetres in diameter. On pouring mercury into the longer limb no escape of air took place through the cork disc, even when there was a considerable difference in the level of the mercury in the two limbs of the tube, and the position of the mercury even after some time was the same as at the beginning of the experiment. Similarly, as Wiesner showed, all other kinds of tissue refuse to permit filtration of gas under pressure, if they are completely closed on the outside. In many cases it is convenient, in making experiments on this subject, to have the bent glass tube (5–6 mm. in diameter) provided with a metallic attachment, such that a suitable object can be inserted into it, and fixed air-tight by screwing on a perforated cap. To protect the tissue it is laid between rubber washers. Suitable objects for investigation are the skin of an apple, the seed-coats of peas and beans, and pieces of living or dried ivy-leaf, whose upper surface as is known is free from stomata.

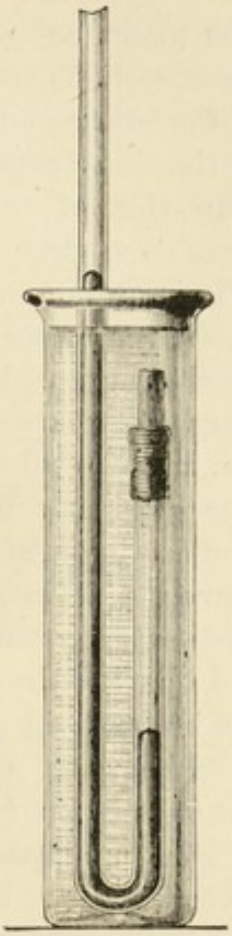

Fig. 63.—Apparatus for determining the permeability of the wood vessels to air.

Before going on to consider the negative pressure of the gases in the elements of the wood, we will here proceed to make a few experiments regarding the permeability to gases of the lumen of the wood vessels, and also of the membranes of the elements of the wood. If pieces of twig, about 6 cm. long and 8 mm. thick, provided with cortex, are fixed by means of rubber tubing in the shorter limb of a bent glass tube (see Fig. 63), and we then pour mercury into the longer limb of the tube, and place the apparatus in a cylinder of water so that the upper cut end of the stem is a few centimetres below the surface of the water, we see numerous bubbles of air rise from the smooth cut surface, indicating that the lumen of the vessels is permeable to air. The mercury sinks more and more in the longer limb, rising in the shorter one, till the difference in level in the two limbs is reduced to, say, one or two centimetres. When the mercury has come to rest, the

water which has penetrated into the wood vessels from outside (even into the widest ones), and is there retained by capillary attraction, holds in equilibrium the final mercurial pressure. It is also easy to see why the ultimate mercurial pressure will be less if we experiment, *e.g.*, with stems of Vitis, than if we use stems of Sambucus, Prunus, or Cratægus. The vessels in the first plant, as we can easily ascertain by examination of transverse sections under the microscope, are much wider than those of the others, and therefore also in the Vitis stem the resistance of the water retained by the vessels by capillary attraction is less than that of the water which has penetrated the Sambucus, Prunus, or Cratægus vessels.[2]

It is also of interest to satisfy ourselves that the length of the vessels or segments of vessels is not nearly so great as we have been accustomed to suppose. From a shoot of Alnus glutinosa, about five years old, we isolate under water a middle piece about 7 cm. long. With the upper end of the piece of twig (that, viz., which was originally directed towards the apex of the shoot) we now connect by means of rubber tubing a glass tube 6 cm. long, and connect this in turn with the air-pump. The lower end of the piece of twig we dip into a fluid consisting of 3 parts of water and 1 part of the officinal Liquor ferri oxychlorati.* On exhausting, the brown fluid penetrates into the opened vessels. In the glass tube, however, appears a perfectly colourless fluid. We continue the exhaustion for about quarter of an hour, and then, by means of garden clippers, cut away a portion from the lower end of the piece of twig. If, continuing the injection, the fluid passing into the glass tube still remains water-clear, we cut off another piece, and so on, till finally the brown fluid begins to appear in the tube. This happens in the Alnus twig when it has been reduced to a length of about 5·5–6 cm.

The Ferric oxychloride is highly colloidal, and therefore cannot traverse plant membranes. If accordingly, with a long piece of branch, the Ferric compound does not appear in the tube, its progress in the vessels must have been stopped by a membrane (transverse wall), and the occurrence of such imperforate transverse walls is in this way established. At the same time the injection method affords a means of determining the length of the vessels. The length is obviously that of the piece of twig when the iron

* This fluid is an aqueous solution of Ferric oxychloride.

solution begins to appear in the glass tube. The values obtained always refer to the longest of the vessels present. In three-year-old shoots of Corylus avellana the length is about 11 cm.; the vessels in Aristolochia twigs are very long, often as much as 200 cm. (six-year-old shoot).

We now treat in exactly the same way a 10 cm. piece of Alnus twig several years old. When this, after continued exhaustion, is sufficiently injected with the iron solution, we dip the lower end of it into a mixture of 1 part of commercial ammonia and 3 parts of water, and exhaust further. By microscopic investigation of longitudinal sections, and by comparing successive transverse sections, we determine up to what height the red-brown precipitate thrown down by the ammonia solution reaches in the vessels. At these limits are also to be seen, especially in tangential sections, the oblique, imperforate, division walls of the vessels.[3]

It is a fact of great importance that the membranes of the elements of the wood are very highly impermeable to air, even under considerable pressure.[4] This fact is of high significance for the theory of the negative pressure of air in wood, and also for the theory of the movement of water in plants, and it gains a very special interest, if we place it side by side with the fact, which we shall establish later, that the same wood substance which is penetrated with such difficulty by air, offers scarcely any resistance to the movement of water.

We can best satisfy ourselves that the wood substance does offer very great resistance to the passage of air by using pieces of wood, a few centimetres long, and about the thickness of a finger, cut out from the youngest annual rings of newly felled trunks of Taxus baccata or Abies pectinata. Such a piece of wood is fixed air-tight in the shorter limb of a bent glass tube by means of rubber tubbing. We then pour mercury into the longer limb, and place the apparatus in a glass cylinder filled with water (see Fig. 63). If we use fresh pieces of wood, or still better fresh pieces of wood which have been lying for some time in water, a pressure of 76 cm. of mercury is often insufficient to force air through. We shall see in another place that the tracheides of the wood do not directly communicate with each other. We shall also see that water can filter with the utmost ease through the closing membranes of the bordered pits of the tracheides, while air cannot traverse them even under high pressure. If we find that by

means of the apparatus above described we can at a considerable pressure, or even at a comparatively small one, force air through pieces of coniferous wood—and this result we do sometimes obtain —this always indicates the presence in the object either of very long tracheides (*e.g.* in the medullary sheath) or of intercellular spaces, which, indeed, as Russow has shown, are not completely absent even in coniferous wood. In an experiment with a piece of Taxus baccata stem, deprived of its cortex, and about 50 mm. long and 6 mm. in diameter, I found that air escaped from the upper cut surface at a pressure of 20 cm. of mercury, but with a pressure of 15 cm. there was no longer any escape of air.

We may also employ the poroscope of Christiani to demonstrate that the tracheides of the wood are impermeable to air under a certain pressure. The apparatus, which is made of glass, is shown in Fig. 64, and, as we see, consists essentially of two manometers. If a short peeled piece of a shoot of Taxus or Abies is inserted between *a* and *b*, and fixed air-tight by means of sealing-wax, and we now blow into the end of the tube *Sch*, the height of the mercury in the manometer *M* is of course considerably changed, but not that of the mercury in the manometer *M'*, since no air passes through the wood. If we now experiment with the vascular wood of a dicotyledonous plant, the height of the mercury is naturally at once altered in both manometers on blowing, since the vessels provide an open communication between *a* and *b*.

Another important fact, which can be demonstrated in lecture by the following experiment, is that there is no open communication between the gases in the cellular and intercellular spaces. We experiment with branches of Cornus mas, Philadelphus, and Syringa, using pieces 30 cm. long and 1 cm. thick, and provided with numerous lenticels. The object is fixed in the shorter limb of a bent glass tube, so as to project into it for about two-thirds of its length. The end which is to be introduced into the tube is first of all made air-tight with sealing-wax. We now place the apparatus in a large glass cylinder full of water, and pour mercury into the longer limb of the tube. If we do not employ too high a mercury pressure, say about 20–30 cm., air escapes at the upper cut surface of the object only from the cortex, proving that no free communication exists between the intercellular spaces on the one hand and the vessels on the other. If we raise the pressure, air escapes also, especially after a little time, from the wood, since

under such conditions air is forced into the intercellular spaces, which, as recent researches have shown, are not entirely absent even in the wood, though they are there certainly very narrow.[5]

It is even possible moreover, at any rate in some plants, that air can at high pressure pass in small quantities from the intercellular spaces through the membranes of the vessels (gas filtration).

It is also instructive to investigate the phenomena under consideration with the help of the apparatus depicted in Fig. 65,

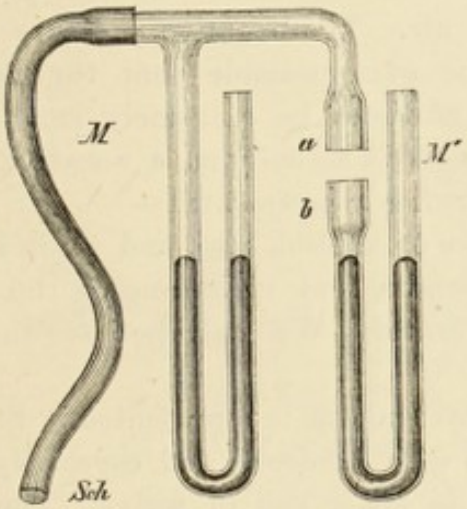

FIG. 64.—Poroscope.

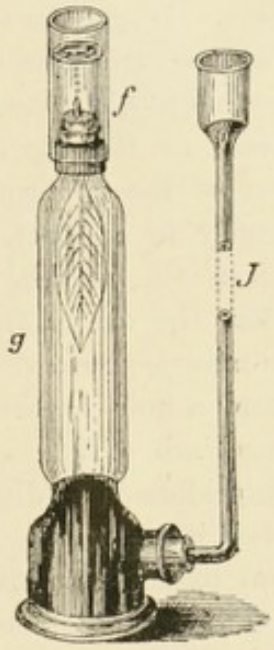

FIG. 65.—Apparatus for investigating the movement of gases in the plant. (After Pfeffer.)

using leaves of Heracleum, Ægopodium, etc., or shoots of various plants (*e.g.* of Prunus Padus). The glass cylinder *g* is closed with a cork, in which the leaf-stalk or shoot-axis has been cemented air-tight. The cylinder *f*, which contains water, may fit on the same cork. The cut surface of the object lies just below the surface of the water, so that it can be observed through a suitably mounted microscope magnifying twenty or forty times. If now mercury is poured into the tube *J*, so as to compress the air in *g*, which it is best to keep moist by means of a layer of water on the mercury, the air enters the stomata and escapes at the cut surface, as the microscope shows, only from the cortex and pith. If we increase the pressure, small quantities of air may also

escape from the vessels. It is finally very instructive to fill *g* with Carbon dioxide, and *f* with clear lime-water, and then proceed as before. It is found that the Carbon dioxide forced into the stomata escapes from the cut surface, and renders the lime-water turbid.

We now proceed to acquaint ourselves in detail with the facts which are known concerning the negative pressure of the air in the wood-vessels. It is certain that at particular times, when the transpiration is feeble, the vessels of the wood contain larger or smaller quantities of water, viz. in spring, and also in summer at night-time. When the transpiration becomes energetic, the water is used up, and since, as we have seen, wood substance is extremely impermeable to air, a negative gaseous pressure will be set up in the vessels, *i.e.* the air in the vessels will be at a lower pressure than that of the atmospheric air. The negative pressure varies considerably in amount. It is even possible that the gaseous pressure in the vessels may at times be extremely small. To prove that the air in the vessels is actually at a negative pressure, we may perform the following experiments :—

We bore a hole to the centre of a birch tree, and fix in it, air-tight, one limb of a glass tube, bent at right angles, the other limb being allowed to dip into water. We shall soon see that the water rises in the tube.

We put together an apparatus such as is represented in Fig. 66. A cut shoot (I experimented with Lonicera, and especially with willow twigs) is placed with its lower end in water. The bent tube *G* is in air-tight connection with the branch, say at *a*, by means of rubber tubing, its other end dipping into water or mercury. Owing to the rarefaction of the air in the vessels, brought about by transpiration, the fluid soon rises in the tube, as in the previous experiment.

For further experiments we may use shoots of Ampelopsis, Vitis, Clematis, Aristolochia, Phaseolus, Helianthus, Quercus, Robinia, or Juglans, without, however, removing them at first from the parent plants. The bean plants may be grown in flower pots. In experiments with Ampelopsis, Quercus, and Juglans we test the behaviour of branches several years old. We bend these down, dip them into an aqueous solution of eosin at a point about 50 or 100 cm. from the end of the branch, and then sever them at this point, under the solution, with shears. The cut surfaces are left in the solution for two minutes longer. We then carefully

wash them, and examine both the isolated portion of the branch, and that still in connection with the parent plant. It is found that the solution has penetrated many centimetres, as is indicated by the staining of the wood, whether the plant be herbaceous or woody. The experiments succeed particularly well, as I have

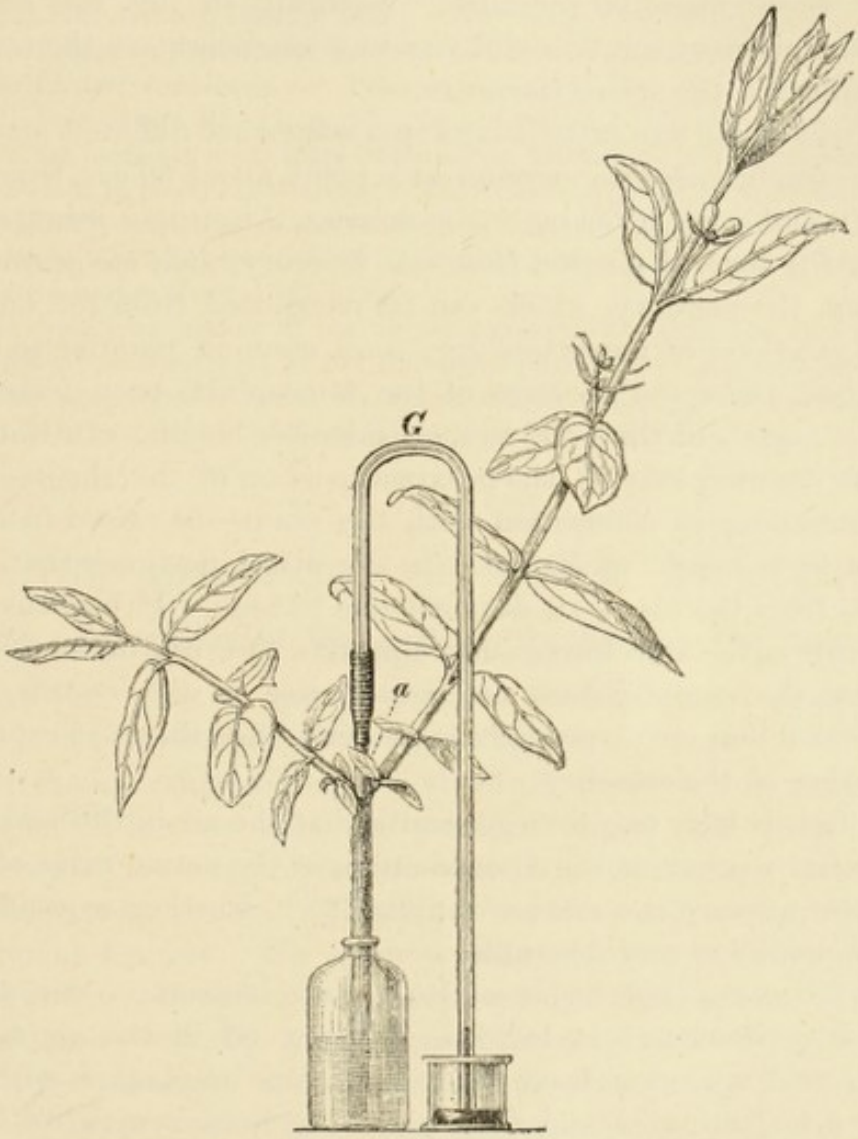

FIG. 66.—Apparatus for proving the negative pressure of air in the vessels.

often found, with Ampelopsis and Phaseolus shoots, and yield particularly striking results when we work with potted bean plants which have been allowed to wither to some extent, or shoots of the wild vine cut through on a very hot, dry summer day.

These experiments prove that the air in the vessels is at a negative pressure, for the considerable rise of the coloured solution

in the stems can only be due to the atmospheric pressure forcing the fluid into the vessels. The rise of the eosin solution is not merely the result of capillarity, as may readily be proved. If we sever shoots of the plants in air, and then quickly dip their ends into the eosin solution, it rises but little in the vessels, because air has penetrated into them, and their gaseous contents are no longer under negative pressure. Naturally in this last experiment the pigment solution will rise to a less height in the vessels by capillarity, the wider these are.

We now make our experiments in a somewhat different manner. We dip the branch into mercury at a point about 50 cm. from the summit, and sever it below the mercury. After two minutes we remove the severed portion from the mercury, and, on peeling it, find that the mercury, which can be recognised from the outside by the presence of numerous grey lines running parallel to each other, has, under the pressure of the atmosphere, been forced up into the vessels of the wood to a considerable height. In Robinia, *e.g.*, the mercury may in the proximal portion of the shoot—that, viz., remaining in connection with the plant—be raised in single vessels to a height of 50 cm. In the distal portion—that, viz., severed from the plant—it does not rise so high. If branches cut in the air have their lower ends dipped a few centimetres below mercury, the mercury does not usually rise in the vessels, since the upward mercurial pressure is generally less than the capillary depression of the mercury.

While it is easy to prove generally that the air in the vessels is at a negative pressure, the determination of the actual value of this negative pressure is a matter of difficulty.[6] Further experiments on this point are very desirable.

The following experiment is also a very instructive one, and it is readily performed in lecture. We cut off in the air a long branch of Ampelopsis, leave the cut surface in contact with the air for a few minutes, and then place the branch with its lower end in water. After twenty-four hours we bend the shoot into eosin at a point about 10 cm. from the first cut surface, and sever it under the fluid. The solution rises to a considerable height in the vessels, from which it follows that under the conditions described, negative gaseous pressure in the vessels is after a time again set up in branches which have been cut off in the air. This may be due to a variety of causes.[7]

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[1] See Wiesner, *Sitzungsberichte d. Akad. d. Wiss. zu Wien*, 1879, Abth. 1, Bd. 79, Aprilheft, and the same, Bd. 98, p. 670. See also Lietzmann, *Flora*, 1887, Bd. 70, and Mangin, *Extrait des Ann. de la Science agronom. franç., etc.* T. 1, 1888.

[2] See Sachs, *Handbuch d. Experimentalphysiologie d. Pflanzen*, 1865, p. 250.

[3] See especially Adler, *Inaugural-Dissertation*, Jena, 1892, and also Strasburger, *Histologische Beiträge*, Jena, 1891, Heft 3, p. 510.

[4] See Sachs, *Arbeiten d. botan. Instituts in Würzburg*, Bd. 2, p. 324, and M. Scheit, *Botan. Zeitung*, 1884, p. 180.

[5] See Höhnel in Pringsheim's *Jahrbücher*, Bd. 12, p. 72.

[6] See Pfeffer, *Handbuch der Pflanzenphysiologie*, Bd. 1, p. 109, and von Höhnel, *Jahrbücher*, Bd. 12, p. 99. In comparative researches on negative pressure, far more attention must be paid than hitherto to the length and width of the vessels, to possible variations in the width of a vessel at different points, to the capillary resistance of the vessels, and other conditions. It would carry us too far to take all these matters into consideration here. See also especially Adler, *Dissertation*, Jena, 1892, p. 42.

[7] The conditions relating to the negative pressure of air in the vessels have been especially investigated by von Höhnel (Haberlandt's *Wissenschl.-praktische Untersuchungen auf dem Gebiete des Pflanzenbaues*, Bd. 2, pp. 89 and 120), and Sachs (*Arbeiten d. botan. Instituts in Würzburg*, Bd. 2, p. 168).

V. THE ABSORPTION OF WATER BY PLANTS.

69. Absorption of Water from the Soil by the Roots.

When the soil is more or less moist, the individual particles of which it is composed are surrounded by coats of water. We may picture them as surrounding the elements of the soil in concentric layers, and it is clear that the particles of soil will retain more energetically those molecules of water in direct contact with them than those more remote. This is in fact the case, as the following experiment teaches. We grow a bean plant in a flower pot in good garden soil, and when the primordial leaves have attained a considerable size, the plant is put in a place where it is screened from direct sunlight and can only transpire feebly. From this time we supply no more water to the soil, so that the plant gradually withers. When it has become very limp, we take small samples, a few grams in weight, from parts of the soil traversed by numerous roots, and accurately determine the quantity of water they contain by drying at 100° C. I found, in experiments not made with Phaseolus certainly, but with Cucurbita, that the humous garden soil employed, still contained, after the plant had withered, more than 15 per cent. of water; and we thus see that a part of the water of the soil is held very firmly by its constituent particles.

The plants wither under the conditions described, because the roots are unable to take up this firmly retained water quickly enough, and in sufficient quantity, to cover the loss by transpiration.[1]

It is further instructive to remove from the flower-pot small quantities of soil, a few grams in weight, after the plants have withered considerably, and determine their behaviour when placed in air containing much aqueous vapour, but not so moist that dew will be deposited. It is best to place the weighed samples of soil in small dishes, together with a vessel of water and a hygrometer, under a box. The air in the box will obviously be very rich in aqueous vapour, and the mercury in the dry-bulb thermometer of the hygrometer will consequently stand only slightly higher than that of the wet-bulb thermometer. In spite of the large quantity of water in the air surrounding the samples of earth, it is found that they do not condense any of the vapour, but, on the contrary, continue to lose water by evaporation. We know that thoroughly dry earth is able to condense vapour, but this property of the soil is of no importance for vegetation in general, since most plants, as our experiments teach, perish when the soil still contains so much water that its power of condensing aqueous vapour, even under very favourable conditions, cannot yet be exhibited at all.

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[1] See Detmer, in Wollny's *Forschungen auf dem Gebiete der Agriculturphysik*, Bd. 1, and *Journal f. Landwirthschaft*, 27. Jahrgang. There is also given the literature, and further features of the absorption of water by roots are discussed.

70. Absorption of Water by the Leaves.

The question of water absorption by leaves is not of great physiological interest, but still it may be here briefly discussed. In many leaves (Brassica, Zea, Aristolochia Sipho, etc.) on dipping the lamina into pure water it appears to be covered by a silvery layer, which is only interrupted along the course of the stronger nerves. On removing the leaves from the water, we find that only the cuticle over the nerves, and hairs which may be present, have been wetted. The cuticle over the mesophyll, owing to its more or less pronounced waxy character, cannot be wetted, and hence remains dry even after contact with the water. The silvery lustre referred to arises from a layer of air between the leaf-tissue and the water, whereby the light is totally reflected. If the leaves

are left in water for some time, their surface becomes wet, and then also the silvery lustre disappears.[1] When the leaves are thus left for a long time under water, the water can undoubtedly penetrate into the plant through the cuticle (but also in other ways). If weighed leaves, in absence of light, are dipped with the blade in water, while the cut surface of the leaf-stalk, which may suitably be cemented with wax, remains unwetted, it is in fact found that the leaves, taken after a time out of the water and very carefully dried with blotting-paper, now weigh more than at the beginning of the experiment. Naturally, this can only take place when we experiment with leaves whose cells do not beforehand exhibit their maximum turgescence. I obtained specially good results when I dipped leaf-blades of Coffea arabica or Syringa vulgaris in water for a shorter (three hours) or longer (twenty hours) time, and when the leaves had been kept in a shady place for two hours before making the experiment, so that they were not too rich in water.[2]

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[1] See Sachs, *Handbuch d. Experimentalphysiologie d. Pflanzen*, p. 159.

[2] See Detmer in Wollny's *Forschungen*, Bd. 1, Heft 2, and *Journal f. Landwirthschaft*, 27. Jahrgang, p. 105.

71. Some Movements in Plant Structures Related to Their Absorption of Water.

The inner leaves of the involucre of Carlina acaulis, a plant which grows on dry calcareous soils, show particularly interesting phenomena due to absorption of water. If the whole inflorescence is moistened, all the inner leaves of the involucre lay themselves together (see Fig. 67); drying causes them to spread out again (see Fig. 68). The involucral leaves possess a silvery white colour, except along the middle of the under side, where they are coloured brown. If a single involucral leaf be removed from the inflorescence of Carlina, and this brown region be moistened with water, a movement immediately takes place, and the under side of the leaf rapidly becomes convex in form. We prepare delicate transverse sections through the middle part of an involucral leaf, and note the following details of structure. An epidermis is present on the upper and lower surfaces. The epidermal cells of the under side are coloured brown. The bulk of the tissue enclosed by the epidermis is composed of parenchyma, which is traversed by a few

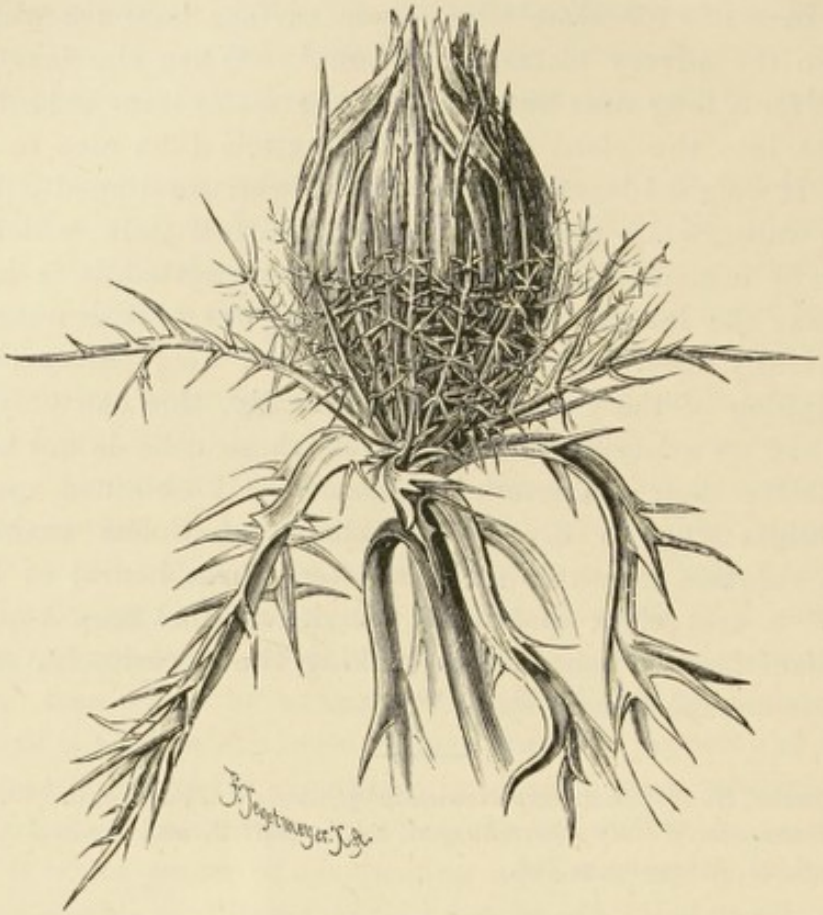

FIG. 67.—Carlina acaulis, inflorescence with closed involucre

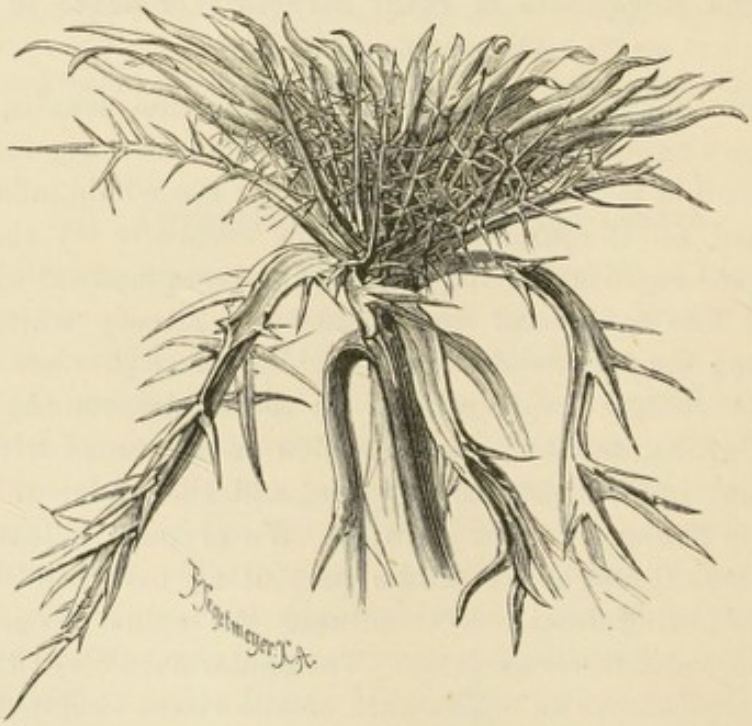

FIG. 68.—Carlina acaulis, inflorescence with expanded involucre.

vascular bundles. The parenchyma of the lower side does not border directly upon the epidermis, being separated from it by a layer of sclerenchyma, consisting of about three layers of strongly thickened elements arranged side by side without interspaces. This sclerenchyma it is which causes the movements described, since its constituent elements elongate very much more than those of the parenchyma when the leaves are moistened, and *vice versâ* shorten much more when the leaves are dried. The movements exhibited by the involucral leaves of Carlina are not directly dependent on the vital activity of the cells; the leaves are also capable of movement when dry and dead.[1]

If we observe plants of Carlina in their natural habitats, we shall at once see that in damp, rainy weather their inflorescences are shut, the involucral leaves having closed together in consequence of being wetted, so that they protect the flowers against the injurious effects of the weather. Characteristic movements due to absorption of water, but just as little directly associated with the life of the cells as those of the involucral leaves of Carlina, are to be observed in the beaks of Erodium fruits, and in the awns of Stipa. In Fig. 69 is depicted a dry mericarp of Erodium with its beak. The lower part, *s*, of the beak is spirally twisted, but not the upper part, *s'*. We lay such a fruit for a short time (about half a minute) in water, and then stick it with its lower end in loose moist sand, and cover with a bell-glass. The movement which takes place is readily seen, and in the moist air it continues till the lower part of the beak is unwound, and the entire beak is stretched straight. If we stick a moistened Erodium fruit into moist sand, and by means of a vertically placed piece of wood prevent the movement of the beak, which when dry is directed almost horizontally, the induced movement is transferred to the fruit, which being acted on by a vertical pressure is screwed into the sand. In nature the fruits of Erodium do actually bury themselves in the ground by means of the characteristic power of movement of their beaks.

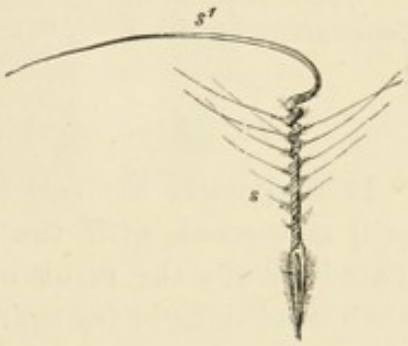

FIG. 69.—Erodium fruit.

Fig. 70 depicts the fruit and awn of Stipa. In the dry fruit the lower part, *e*, of the awn is spirally wound. The part *k* of

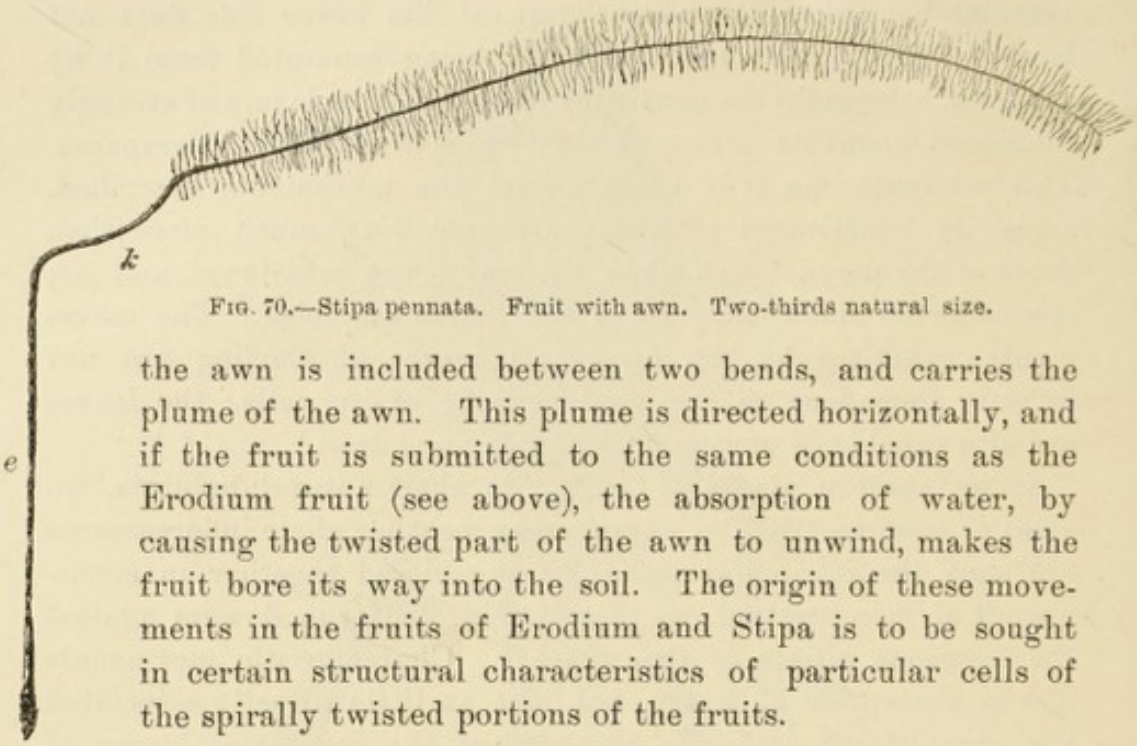

FIG. 70.—Stipa pennata. Fruit with awn. Two-thirds natural size.

the awn is included between two bends, and carries the plume of the awn. This plume is directed horizontally, and if the fruit is submitted to the same conditions as the Erodium fruit (see above), the absorption of water, by causing the twisted part of the awn to unwind, makes the fruit bore its way into the soil. The origin of these movements in the fruits of Erodium and Stipa is to be sought in certain structural characteristics of particular cells of the spirally twisted portions of the fruits.

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[1] See Detmer, *Journal f. Landwirthschaft*, 27. Jahrgang, p. 110, and Räthay, *Sitzungsberichte der Akad. d. Wiss. zu Wien*, Bd. 83.

72. Absorption of Water by Fruits and Seeds.

It frequently happens that juicy fruits (stone fruits, berries), still connected with the parent plant, in rainy weather burst. This is chiefly the result of the almost complete cessation under such conditions of transpiration. The cells of the parenchyma of the fruits turgesce very strongly, tensions are set up in the fruit tissue (the epidermis in particular becomes violently stretched), and finally the fruits burst. Another factor may in a subordinate way co-operate in bringing about this result, viz. the absorption of water by the fruits, whereby the turgidity of the parenchyma cells is still further intensified. To prove that such absorption actually takes place, uninjured cherries or grapes are accurately weighed and laid in water with the fruit-stalks outside. After four to eight hours they are taken out, carefully dried, and again weighed. Generally it will be found that they have increased to a not inconsiderable extent in weight.

We lay a few wheat-grains in water. After about twelve hours the process of soaking will already be far advanced, and the grains will have become soft. The thin skin surrounding the

wheat-grain readily permits the water to penetrate into the inside of it. To acquaint ourselves with the anatomical structure of the fruit-coat and seed-coat of the wheat, we make the sections, which must be as thin as possible, from grains which have not been in water too long, and treat them before observation with potash solution. Fully soaked grains are unsuitable, since it is very difficult to prepare good sections from them. The potash causes the tissues to swell up, so that the details of structure of the

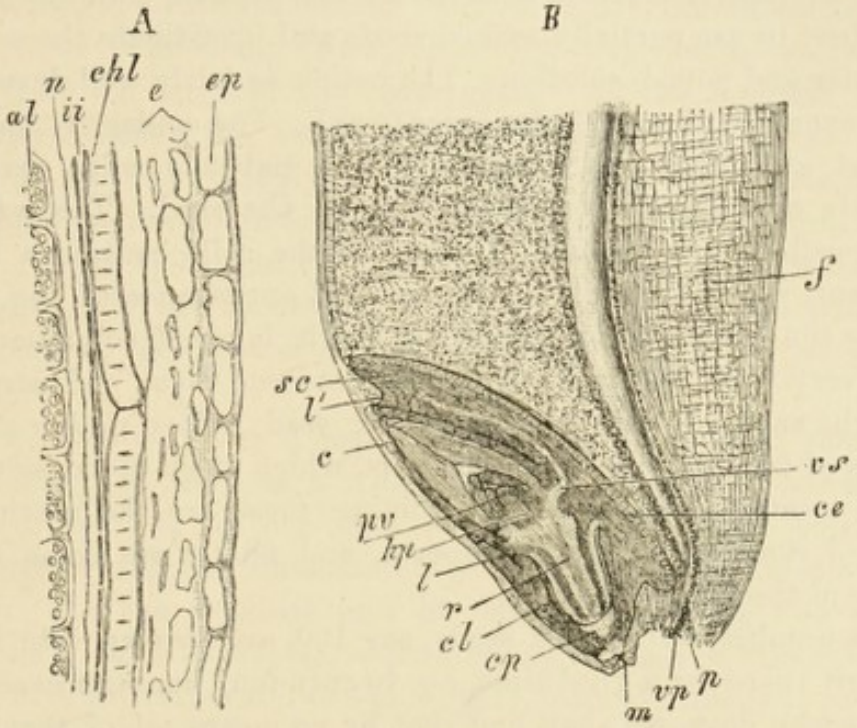

FIG. 71.—Triticum vulgare. *A*, transverse section through the coats; *ep*, epidermis; *e*, adjoining layers; *chl*, chlorophyll layer: these all belong to the fruit-coat; *ii*, derived from the inner integument; *n*, the outermost layer of the nucellus: these together form the seed-coat; *al*, aleurone grain layer of the endosperm. Magn. 240.

B, medium longitudinal section through the lower part of a ripe fruit. To the left, below, the embryo, with *sc*, the scutellum; *l'*, its ligule, and *vs*, its vascular bundle; *ce*, its cylindrical epithelium; *c*, sheathing part of the cotyledon; *pv*, vegetative cone of the stem; *hp*, hypocotyl; *l*, ligule; *r*, radicle; *cp*, root-cap of the radicle; *cl*, root-sheath; *m*, point of emergence of the radicle, corresponding with the micropyle of the ovule; *p*, the fruit-stalk, and *vp*, its vascular bundle; *f*, side wall of the fruit. Magn. 14. (After Strasburger.)

fruit-coat and seed-coat become fairly distinct (see Fig. 71). The fruit-coat consists of the cuticularised epidermis, *ep*, a layer of parenchyma, *e*, and a wide layer adjoining these, whose elements are tangentially elongated, *chl*. The seed-coat is composed of several layers, but the cellular structure of the outer layers, *ii*, cannot be properly made out, and they present themselves for the most part merely as brown strips. The innermost layer, *n*, of the seed-coat, which lies immediately below these brown strips

consists of transparent cells. The seed-coat encloses the endosperm and the embryo. To these we return later. It may here be observed merely that the layer of the endosperm immediately within the seed-coat (*Kleberzellschicht*) consists of a single layer of almost quadratic cells—which, though this, it is true, cannot be made out in the sections treated with potash solution—contain aleurone grains (no starch). Then come the more or less starchy tissues of the endosperm.

We prepare transverse sections of the seed of Lupinus luteus. It is best to use partially soaked seeds and investigate the sections in water and potash solution. The cuticle is fairly well developed, and covered with a granular layer (wax). The epidermis demands special attention. It consists of long palisade cells, arranged radially with respect to the surface of the seed. The walls of these cells are very thick. Groups of the cells contain a brown pigment, which accounts for the spotted appearance of the seeds. Below the epidermis we observe a single layer of columnar cells, with very wide intercellular spaces between them, and arranged at right angles to the surface of the seed. Then follow several layers of tangentially elongated cells, which swell up considerably when treated with potash. Adjoining these are the much compressed remains of the endosperm, and finally we come to the tissue of the cotyledons.

If a number of Lupinus seeds, say 100, are thrown into water, and left there for a good time, *e.g.* twenty-four hours, or even eight to fourteen days, we shall find that by no means all of them have absorbed water. This resistance to the influence of water, which is of great biological significance, is characteristic of many other kinds of seeds besides those of Lupinus, and in the case before us is due to the fact that the cells of the perfectly intact palisade layer of the seed-coat, owing to special peculiarities of their membranes, are only with great difficulty penetrated by water. If we injure the palisade layer of a lupin seed, it always soaks readily when placed in contact with water.[1]

The seeds of Pisum sativum are among those that soak readily, as we can satisfy ourselves by putting them in water. The testa of the Pisum seed in many ways resembles in its structure that of Lupinus. We peel the seed-coats from soaked seeds, fold them up, and prepare delicate transverse sections, which we examine in caustic potash. Here also the palisade layer is followed by a columnar layer. Then comes a many-layered parenchyma, the

cells of which are tangentially elongated, and lastly the remains of the compressed endosperm.

The soaking of most seeds is effected by imbibition and osmosis. In some cases, *e.g.* the seeds of Linum usitatissimum, the process is accelerated by the formation in contact with water of a mucilage, which very energetically attracts and retains the water. In fact, each Linum seed at once surrounds itself, when brought into contact with water, with a sheath of mucilage. We prepare very delicate transverse sections of dry Linum seeds, and mount them in alcohol. Now, observing the result, we run in water from the edge of the cover-glass. At the moment when the water reaches the section, the epidermal cells of the seed-coat swell up very strongly, and exhibit the mucilage present as thickening layers in their outer walls, the cell-walls remaining intact. These epidermal cells, as we now easily see, are arranged perpendicularly to the outer surface of the seed. We will not here consider further the complicated structure of the seed-coat of Linum.

If we examine a dry Phaseolus seed, and place it so that the hilum, which appears as a white strip, is directed towards the observer, we find at one side of the strip a small hole, the micropyle, which lies immediately over the tip of the root of the embryo. At the opposite end of the hilum we see two small swellings (*Doppeltuberkeln*), separated by a shallow commissure. These, with the hilum and the micropyle, form the hilary apparatus of the seed. The micropyle plays a particularly important rôle in the absorption of water by bean-seeds, as the following experiment shows. One Phaseolus seed, *a*, is completely immersed in water. A second seed, *b*, as nearly as possible equal to *a* in weight, is fixed in a suitable manner on a needle, and placed in water in such a way that the hilary apparatus is not wetted. If we weigh after a few hours, it will be found that *a* has taken up a comparatively large quantity of water, while *b* has absorbed but little.[2]

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[1] See Detmer, *Journal f. Landwirthschaft*, 27. Jahrgang, p. 119.

[2] On the structure of seed-coats, see especially Sempolowski's *Dissertation*, Leipsic, 1874. There also the most important literature is given. See also Mattirola and Buscalioni, *Memorie della R. Accademia delle Scienze di Torino*, Serie II., T. xlii.

73. Further Experiments on the Absorption of Water by Seeds.

The quantity of water which has been absorbed by different kinds of seeds, when fully soaked, is by no means the same. The capacity for absorbing water is therefore different in different kinds of seeds. We determine the weight of a few air-dry wheat-grains and peas, and then put them in water for twenty-four hours. At the end of this time we dry the seeds well, and again determine their weight; then return them to the water and once more weigh after six hours. Further weighings may need to be made, but at all events it will be found that, when the weight of the seeds has become fairly constant, the peas contain more water when fully soaked than the wheat-grains. Peas take up about 100 per cent. of their weight of water, wheat grains only 40 to 60 per cent.

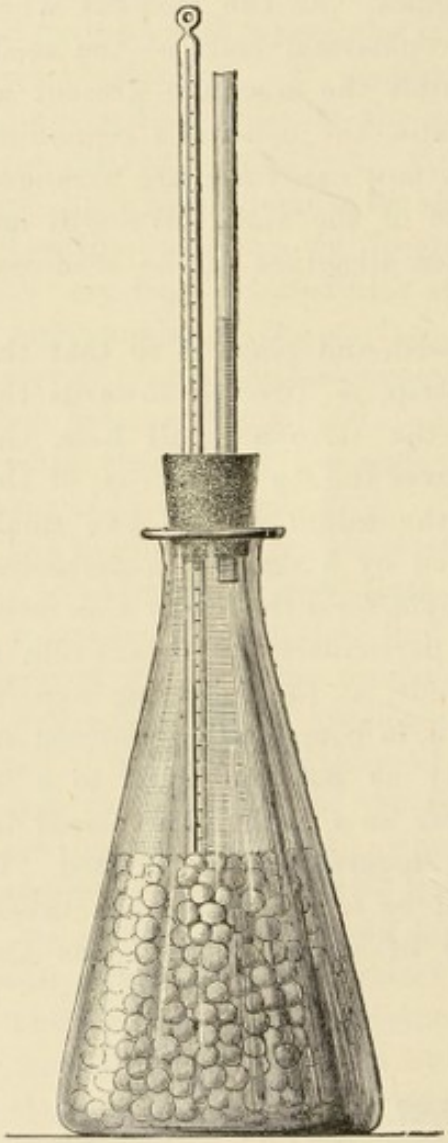

FIG. 72.—Apparatus for observing the phenomena of absorption of water.

In many cases (*e.g.* beans, peas) investigation at once proves that the volume of the seeds, when they have been soaked, is much greater than when they are air-dry. But the question also arises whether the total volume of seeds and water is changed in the process of soaking. The following experiment may be made to answer this question. We use the apparatus drawn in Fig. 72. In a flask of about 600 c.c. capacity we place 300 gr. of peas (I used white Giant peas), the flask is then completely filled with water and immediately fitted with a two-holed rubber stopper. Through one hole passes a thermometer, through the other a straight glass tube 0·5 cm. in diameter. The position of the water in the latter may easily be determined at

the commencement of the experiment, and subsequently at intervals of five minutes by means of a millimetre scale. We observe that for some time the water rises higher and higher. This goes on for three-quarters of an hour, or sometimes even for an hour and a half. Then the water sinks for a short time, or under some conditions even for a few hours, finally again rising. In exact investigations the influence of temperature on the position of the water must be taken into consideration.

If seeds are placed in contact with water, and imbibition takes place, the water entering the seeds must suffer condensation. Such a process would, however, lead to a diminution of the aggregate volume of seeds and water. Of this, however, nothing is to be seen in our experiment. On the contrary, at the commencement of the experiment, the water rapidly rises in the tube, clearly indicating an increase in the aggregate volume of the seeds and soaking fluid. The causes underlying this are therefore at all events preponderating in their effect on the position of the water, and they are to be sought in a folding of the testa, which takes place in the first stage of the soaking. The testa raises itself from the cotyledons of the seed, and between the cotyledons and the seed-coat are formed cavities filled with rarefied air, so that the aggregate volume of the seeds and the water must necessarily be increased. If, in fact, we employ peas whose testa we have injured, the ascent of the water in the tube, as I have found, does not take place during the whole of the first period of the experiment.

During the second period of the experiment the water sinks in the tube, indicating a decrease in the total volume of the seeds and water, and this must be referred to the penetration of water into cavities of the seeds. I do not here proceed to consider the causes leading to the renewed rise of water in the tube during the third period of the experiment. As to this, and also as to the behaviour of other seeds in this respect, my work cited below is to be consulted.[1]

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[1] See Detmer, *Vergleichende Physiologie des Keimungsprocesses der Samen*, Jena, 1880, p. 71, and Mattirola's work cited below. See also *Botan. Centralblatt*, 1892, Bd. 52, p. 155.

74. Absorption of Water by Mosses.

Mosses possess not true roots, but rhizoids. To acquaint ourselves accurately with these organs we select Bryum cæspiticium, a moss which is frequently met with on moors. Soil clinging to the plants is removed as completely as possible by means of a stream of water. The lower part of the stem is cut off, transferred to a slide, and examined. It is seen to give off long, multicellular, very thick, brown-coloured threads, which possess numerous fine branches, and are only colourless at their ends. The partition walls between the cells are seen to be placed obliquely. The rhizoids, with which we have here to do, have as their special function the task of fixing the plant in the soil. As organs for absorbing water, they possess, at least in many mosses, only a subordinate importance.

If a moist sod of Hylocomium triquetrum is placed in a flat dish containing water, the upper parts of the plants soon dry up. This shows that no energetic and adequate conduction of water takes place in the interior of mosses, such as occurs in the higher plants. If, however, we closely observe the clump of Hylocomium lying in water when the summits of the plants are dry, we find that all the stems are moist for a fairly long distance above the surface of the water. Water is therefore raised by the plants to a certain height, and this takes place by capillary attraction. The water rises to that level in the narrow spaces present between the stems and their closely applied leaves ; but obviously those parts of the plants which cannot be reached by this external water supply must dry up. If a vigorous shoot of Hylocomium triquetrum is well dried between blotting-paper, and is then dipped with its base or apex in a solution of an aniline pigment (I used aqueous solution of methyl violet), we readily see that the water rises to some height by capillary attraction. If clumps of Hylocomium or Hypnum, freed from adherent soil, are weighed when in the air-dry condition, immersed in water for say ten minutes, laid on a sloping glass plate, so that the excess of water may drain off, and then again weighed, it is found that they have been able to take up many times their weight of water. This gives us an idea what large quantities of water may be retained by capillarity by the mosses in a forest ; and, in fact, mosses play an important part in many regions in regulating the hygrometric conditions.

If we examine transverse sections of the stem of Hylocomium

triquetrum, we find that the entire tissue consists of cells, whose walls are yellowish-brown in colour and strongly thickened. The lumina of the peripheral and of a few central elements (which last suggest a central bundle) are much narrower than those of the other cells. In mosses which have no central strand, or only a feebly developed one, composed, as is known, of cells much elongated longitudinally, practically only the external conduction of water through capillarity has to be taken into account. On the other hand, a well-developed central strand appears to offer the possibility of vigorous internal conduction of water, and experiments made with Polytrichum confirm this. A well-developed central strand is in fact present in the stem of Polytrichum, as we may easily ascertain by microscopic examination of delicate transverse sections, and on placing a few stems of Polytrichum formosum close together, with their lower ends in water, I found that even the upper leaves of the plant remained fresh. In this condition the Polytrichum leaves stand off from the stem, while in drying they apply themselves closely to it.

Particular interest also attaches to the manner in which Sphagnums take up water from the outside and retain it. It will here be sufficient to indicate briefly the structure of the leaves of these plants. We select for microscopic examination Sphagnum acutifolium, the green, reddish, or intensely red sods of which are easily found. The mature leaf consists of chlorophyll-containing cells, which are united together to form a network, and of colourless cells, no longer living and containing water or air, which lie between the green cells and have membranes thickened in an annular or spiral manner, and pierced with holes. Through these holes water easily passes into the cells from the outside, and is retained by them, so that a sod of Sphagnum can take up large quantities of water, acting indeed like a sponge.[1]

[1] On the absorption of water by Mosses, see Oltmanns, *Strasburger Inaugural-Dissertation*, 1884.

VI. MOVEMENT OF WATER IN PLANTS.

75. Root Pressure.

If it is desired to prove that by osmosis and turgidity forces can be set up in the cells, which are able to drive the cell-sap through the membranes of the cells, and *e.g.* into the vessels, it will be

convenient to direct our attention first to the phenomena resulting from root pressure. Vigorous pot plants of Cucurbita, Helianthus, Ricinus or Begonia, or willow cuttings well rooted in flower pots, are decapitated, *i.e.* the stem of the plant is cut through at a point a few centimetres above the soil. In winter we may also experiment with pot plants of Sanchezia nobilis (belonging to the Acanthaceæ).* Over the stump projecting from the soil is now

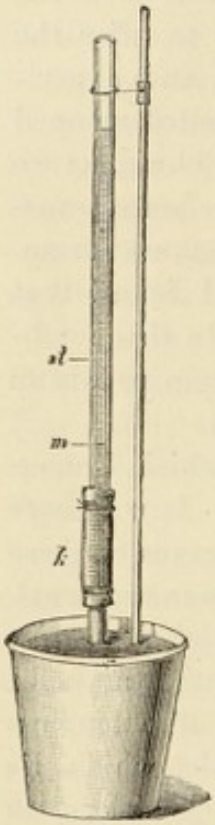

FIG. 73.—Apparatus for experiments on root pressure.

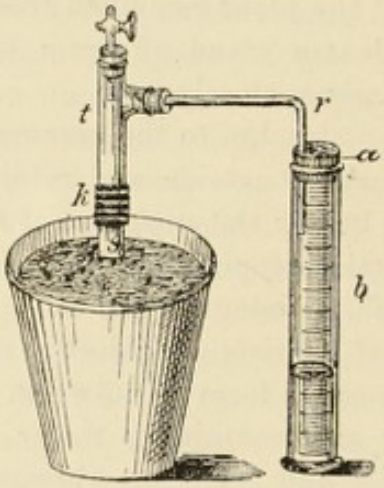

FIG. 74. — Apparatus for experiments on root pressure. (After Pfeffer.)

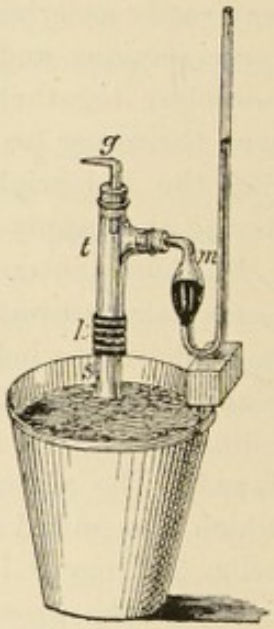

FIG. 75. — Apparatus for researches on root pressure. (After Pfeffer.)

slipped a short rubber tube (Fig. 73), *k*, which serves to connect it with a glass tube, *st*. In order to make the connections air-tight, the rubber tubing is bound over the stem-stump and over the glass tube with thread, or better with elastic. Just above the connection tubing the glass tube is provided with a mark, *m*, easily made with a three-cornered file. On filling the tube up to the mark with water, we shall observe, if the plant had not been transpiring too strongly before the commencement of the experi-

* This plant, which serves exceedingly well, can be readily propagated by cuttings. I found that a plant investigated in January gave sap for fourteen days.

ment, and if the soil in which it is rooted contains plenty of water, that the level of the fluid in the tube soon rises. To demonstrate the outflow of sap from the stem-stump, we may also use the apparatus indicated in Fig. 74. On the stump *s* of the decapitated plant is fixed, by means of a short piece of rubber tubing, a T-shaped glass tube, *t*. In order to make the connection absolutely air-tight, the tubing must, if necessary, be bound with wire. In many cases one is obliged to employ a narrow T-tube. The vertical limb of the T-tube is closed at the top with a cork, through which passes a glass tube provided with a glass stopcock. The horizontal limb is connected up with the outflow tube *r*, which dips down through a loosely fitting cork, *a*, into a graduated measuring cylinder, *b*. The T-tube and the outflow tube must at the commencement of the experiment be filled with water.

To determine the pressure exerted by the sap flowing from the stem-stump, the outflow tube is replaced by a manometer containing mercury, as indicated in Fig. 75. Water is poured into the T-tube, which is then closed with a cork, provided with a short tube, *g*, drawn out to a capillary point. The end of the capillary must be fused up, so that no air is left in the apparatus.

Proceeding in the manner described, we find that in many plants the sap only flows for a few days. I have, however, often observed that the flow may continue for more than a week. If plants are decapitated, and we observe their behaviour without further preparation, we shall find that the sap escapes from the wood, and especially from the vessels.

76. The Flow of Sap from Injured Trees Growing in the Open.

In March or early in April we make a boring in the trunk of a still leafless birch tree, reaching to about the middle of the trunk. In my experiments holes 7 mm. wide were made at a height of about 40 cm. above the ground, where the trees were about 40 cm. in circumference. One limb of a glass tube bent at right angles is cemented air-tight into the boring, while the other limb passes into a flask standing on the ground. Under some conditions a considerable quantity of fluid at once flows from the tree, but in any case it is very instructive to watch the behaviour of the plants for several weeks. Addition of water to the soil by rain favours the outflow of the sap; in the daytime it is usually less than during

the night; or it may be entirely absent during the day, only taking place at night. This is due to the fact that during the day the sap driven into the stem by the roots goes to cover the loss by transpiration, while at night-time at least a portion of the sap can escape, since now the evaporation of water, as the result of external conditions, is usually much reduced. As the season advances and the leaves of the birch unfold, the outflow of sap completely ceases. The transpiration of the tree has now become very considerable.

The observations on the flow of the sap from the birch are to be associated with meteorological observations, in order that we may be enabled to follow closely its dependence on external conditions. We note the character of the clouds, the height of the barometer, and observe the rainfall, the hygrometric condition of the air, and the temperature of the air and of the soil. The necessary apparatus—rain gauge, maximum and minimum thermometers, hygrometer (*e.g.* the well-known wet and dry bulb hygrometer of August)—can all be obtained of Muencke in Berlin. To determine the hygrometric condition of the air from the readings of the August hygrometer, we employ the tables of Jelinek, Vienna, 1876.

If we evaporate birch sap we obtain a residue composed of organic and mineral substances. We can easily ascertain the presence of the latter by evaporating the sap to dryness in a platinum dish, and igniting the residue to decompose the organic substances. The mineral substances remain behind. Fresh birch sap is slightly acid in reaction. On boiling it, a coagulum of proteid material separates out. If we treat a small quantity of the sap with a few drops of Sulphuric acid, and then boil, replacing the water lost by evaporation, we obtain a fluid which, when added to hot Fehling's solution, gives a precipitate of Cuprous oxide. The crude sap contains, viz., cane-sugar, which, by boiling in presence of Sulphuric acid, is converted into grape-sugar, and this exerts a reducing action.

77. The Influence of External Conditions on the Flow of Sap from Decapitated Plants.

We decapitate vigorous pot plants of Cucurbita, Helianthus, Ricinus, or Begonia, and fit the stump of the stem with a tube as in Fig. 73. The soil in the flower pots must not be too moist,

A thermometer is introduced into the soil, which is then covered with tinfoil, as also is the portion of the stem rising above ground. The pots are now placed in a room kept at a very constant temperature, or are introduced into a thermostat. When the soil in the flower pots has assumed the temperature of its surroundings, the observation begins. We note the flow of sap, say, per hour. After several hours, the soil in which the plants are rooted is well watered, and when the temperature has adjusted itself again, the determinations of the flow of sap are continued. Owing to the abundance of water in the soil, the outflow is now found to be much more considerable than before. It must not be forgotten, in this and the following experiments, that the flow of sap in many plants exhibits periodic variations, independent of external conditions (see 78).

The same objects may also be used to prove that conditions of temperature have an important influence on the outflow of sap. The soil in which the plants are rooted is well watered at the commencement of the experiment, and then from time to time the temperature in the thermostat is altered, and in each case, after adjustment of temperature (*i.e.* when the soil has assumed the temperature of its surroundings) the observations on the flow of sap are resumed. At 16° C. more fluid flows out in the unit time (*e.g.* in an hour) than at 12° C. At 20° C. the outflow of sap is more considerable than at 16° C. I find that the optimum temperature in Cucurbita Melopepo is about 26° C. Still higher temperatures retard the flow of the sap, and at a temperature of 43° C. it entirely ceases.[1]

It is frequently necessary—as, for example, in the experiments which we have just been considering—to expose objects for a long period to a constant temperature. We will now describe the methods and apparatus to be employed for the purpose.

First as regards the thermometers, these are to be obtained in a great variety of forms and sizes, and variously graduated, from the Firms named in the Appendix. If we require several thermometers for the same experiment, they must be accurately compared. In special cases, *e.g.* in experiments with the auxanometer, it is of advantage to employ a registering thermometer.

When working with registering apparatus, or with large plants which cannot be introduced into a thermostat, we must take pains to keep the temperature of the laboratory as constant as possible. For small rooms the so-called American stoves, heated with

anthracite coal, are very suitable, while for larger rooms (above 150 cubic metres in capacity) the larger forms of Meidinger stoves are to be recommended. Pfeffer[2] using such stoves succeeded in keeping the variations of temperature, at table height and at some distance from the window, within ± 0°·18 C. for days and weeks.

To expose plants for a considerable time to a temperature of 0° C., we employ the ice-chest described in 49. Places of very constant low temperature, above 0° C., are deeply situated cellars, ice-cellars, and even rooms facing the north in a building with thick walls.

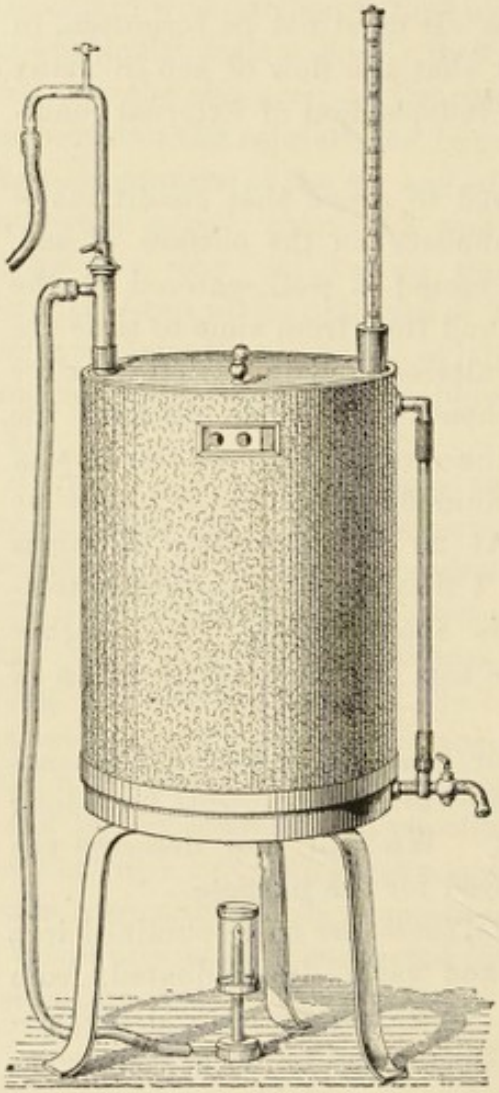

Fig. 76.—Thermostat, with thermo-regulator.

Good thermostats are of very special importance in many physiological researches, *e.g.* in many experiments relating to root pressure. They are to be obtained, *e.g.*, from Dr. H. Rohrbeck, Berlin. (See Appendix.) No. 114 in Rohrbeck's 1891–2 price list is very good; price according to size, 50–200 mks. A simpler, but still very serviceable thermostat is No. 129; price according to size, 20–30 mks. This thermostat is double walled, provided with a water-level gauge and tap, and mounted on a tripod; it has a glass cover, which may be replaced by a bell-glass, and is completely lined with felt. It is best to have it provided with a ventilating arrangement. The apparatus is represented in Fig. 76. Gas pressure regulators, gas-burners, — *e.g.* the convenient micro-burner for very small flames—and thermo-regulators are also to be obtained from Rohrbeck. No. 149 is a regulator which, filled and tested, costs 19 mks. The head of this efficient regulator is made

of metal. In it slides a tube (supply tube), which is put in connection with the gas supply. The regulator is placed in the water space of the thermostat. In many cases, however, it is advisable to let the regulator project into the interior of the thermostat. For temperatures up to 40° C. it is filled with mercury and ether; for temperatures from 40°–70° C., with mercury and alcohol.

In heating up the thermostat, we pull the supply tube as far as possible out of the regulator. As the temperature rises, the ether or alcohol vapour raises the mercury in the regulator, and when the desired temperature is reached, the supply tube is pushed in till the triangular opening at its lower end is just covered by the mercury. The extinction of the flame is prevented by the presence in the supply tube of a fine opening, through which gas can still pass to the burner.

The variations in atmospheric pressure, whose magnitude can easily be determined by means of a barometer, are not without influence on the action of the regulator. Account must be taken of this in using the regulator, if it is desired to keep the temperature in the apparatus constant to a fraction of a degree Centigrade. This is done in the manner described by Rohrbeck in a short paper, which if desired can be obtained from him with the regulator.

In cases in which it is only required to keep the temperature for some time approximately constant, we may also conveniently employ the following apparatus, which moreover, in absence of a hothouse, serves well in winter, especially in cold weather, for rapidly developing seedlings, etc. The thermostat, which rests on a firm stand, consists of a large double-walled box made of strong sheet zinc. The box is about 60 cm. deep, and the same in length and breadth. The space between the double walls of the apparatus may be 3 or 4 cm. This is filled with water, which we admit through an opening in the upper part of the box, while in the lower part of it there is a tap, from which to run off the water when necessary. Through an opening in the double-walled cover of the box passes a thermometer. It remains to be mentioned, that the front of the box consists of a double door. The apparatus is heated by means of a gas flame placed beneath it.

Thermostats should naturally be placed, if at all possible, in rooms as little as possible subject to changes of temperature.

In summer we select rooms with a north aspect, in winter rooms which can be heated with good stoves.

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[1] See Detmer, *Beiträge zur Theorie des Wurzeldrucks*, Jena, 1877, p. 30. See also Wieler, in Cohn's *Beiträge zur Biologie der Pflanzen*, Bd. 6, Pt. 1.

[2] See Pfeffer, *Zeitschrift f. wissenschl. Mikroskopie*, Bd. 7, p. 449.

78. Periodicity of the Root Pressure.

We decapitate pot plants of Cucurbita Melopepo about two months old, or plants of Helianthus tuberosus * about the same age, raised from tubers, or suitable examples of Prunus Laurocerasus. We fit up the stem-stumps with a simple tube or otherwise, as described in 75, cover the soil in the flower pots with tin-foil, to prevent excessive loss of water, introduce a thermometer into the soil, and place the preparations in a room kept as nearly as possible at a constant temperature, or transfer them to a thermostat. If we now determine the quantity of sap escaping during unit time (*e.g.* every hour or every two hours) at different times of the day, we find that the plants, notwithstanding the constancy of the external conditions, do not always yield the same quantity of fluid. The flow of sap in Cucurbita and Helianthus is most active shortly after midday, whilst in Prunus Laurocerasus, according to my observations, the maximum outflow does not take place till towards evening. During the night the flow diminishes. It reaches its minimum in the early hours of the morning, and then begins to rise again. If we have connected the stem-stumps with a simple tube, as in Fig. 73, we must naturally, after each observation, remove any fluid above the mark. This is conveniently effected by means of a thin glass tube. The length of the column of water from the mark to the surface of the fluid, expressed in millimetres, will serve as a measure of the quantity of sap escaping. Comparatively young plants do not, as far as investigations extend, exhibit the phenomenon of periodicity in the flow of sap. For example, in plants of Cucurbita Melopepo one month old, the periodicity has not yet developed.[1] It is obvious that in all experiments special attention must be paid to keeping the temperature as constant as possible.

* The experiments with Cucurbita, Helianthus, and many other plants, may be carried out in the summer. The plants must have been cultivated in a sunny place in the open, or in the cold-house.

In detailed researches concerning the periodicity of root pressure, it is advisable to employ an arrangement for registering the outflow of sap on the principle indicated by Baranetzky (*Abhandl. d. Naturf. Gesellschaft zu Halle*, 1873). The apparatus, with thirty-six glass tubes for collecting the sap, is to be obtained from Albrecht in Tübingen, at a price of 100 marks.

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[1] For the literature, see Detmer, *Lehrbuch der Pflanzenphysiologie*, 1883, p. 122, and Wieler's treatise cited in 77.

79. The Causes of Root Pressure and Related Phenomena.

A glass tube about 80 mm. long and 40 mm. in diameter (see Fig. 77) is covered at one end with a well-washed pig's bladder, then completely filled with a concentrated solution of cane-sugar, and closed at the upper end with vegetable parchment. The tube passes so far through the large cork *K* closing the vessel *Gl*, that its lower end dips into the distilled water contained in the vessel. Over the upper, parchment-covered end of the glass tube is bound a rubber cap, *Kk*, which runs out into a rubber tube, and into this is introduced the twice bent glass tube, *Gr*. When the apparatus has been put together, its action at once begins. Water penetrates osmotically into the tube, closed with membrane at both ends (artificial cell), a pressure is set up in it, since the quantity of water entering is greater than the quantity of sugar solution passing out, and this pressure is after a time great enough to overcome the resistance to filtration offered by the parchment paper. When this is the case, fluid is driven into the tube *Gr*, and it can be collected in the vessel *F*. It is true that the processes leading

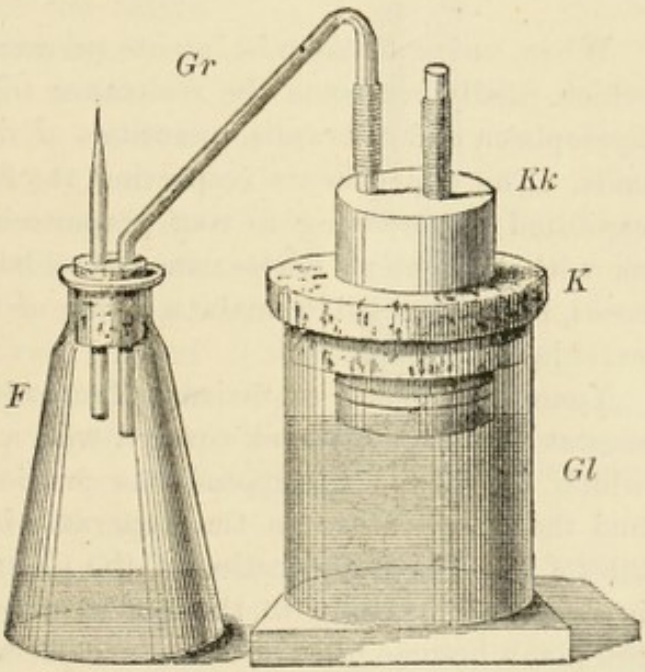

FIG. 77.—Apparatus for illustrating the processes leading to root pressure.

to root pressure and related phenomena are much more complicated than those above indicated, and it is especially to be emphasised, that in the plant cell the protoplasm (and particularly its ectoplasm) plays an important part when pressures are set up in plants by processes of osmosis; but nevertheless the above experiment is of considerable interest in connection with plant physiology. In the cells of the parenchyma of roots, pressures are set up owing to the osmotic capacity of the cell-contents. The strongly turgescing cells finally press out a part of their contents, which passes into the elements of the wood, and is driven up within them.

80. Further Experiments on the Escape of Liquid Water from Plants.

When, owing to osmosis, intense pressures are set up in the cells, which finally overcome the resistance to filtration offered by the hyaloplasm and cell-walls, quantities of fluid are forced out of the cells. The experiments respecting the flow of fluid from the decapitated plants owing to root pressure have already familiarised us with phenomena whose cause is to be sought in osmotic pressures, but there still remain a series of other phenomena which must here be considered.

Young pot plants of maize or Tropæolum are placed in a thermostat (see Fig. 76), and covered with a bell-glass. The soil in which the plants are rooted has previously been well watered, and the temperature in the apparatus is to be kept constant at 20–25° C. The transpiration of the plants under these conditions is almost *nil*. Owing to the root pressure, all the cavities within the plant become filled with water, and water may even escape to the outside. We observe in fact that in the course of from half an hour to two hours, drops of water appear on the tips of the leaves in Zea, and on the edges of the leaf in Tropæolum, which on reaching a certain size fall off and are replaced by new ones. In Zea the water passes out through rifts in the epidermis, in Tropæolum through water stomata.

That pressures as set up, *e.g.*, by root pressure, play an important part in causing the escape of drops of water from leaves is easily demonstrated. To the shorter limb of a glass tube filled with water, we connect a cut shoot of Vitis, Tropæolum, or Impatiens

(see Fig. 78). The cut surface dips into the water. We place the arrangement in a large glass cylinder, pour mercury into the long limb of the tube, and finally cover the cylinder with a glass plate, so as to retard as far as possible the transpiration of the shoot. After a longer or shorter time, drops of water escape from the tissue of the leaves. I saw this formation of drops of water very beautifully in experiments performed as described with shoots of Impatiens Balsamina. With an over-pressure of 35 cm. of mercury, drops of water escaped from the teeth along the margin of the leaves in the course of a few minutes.[1]

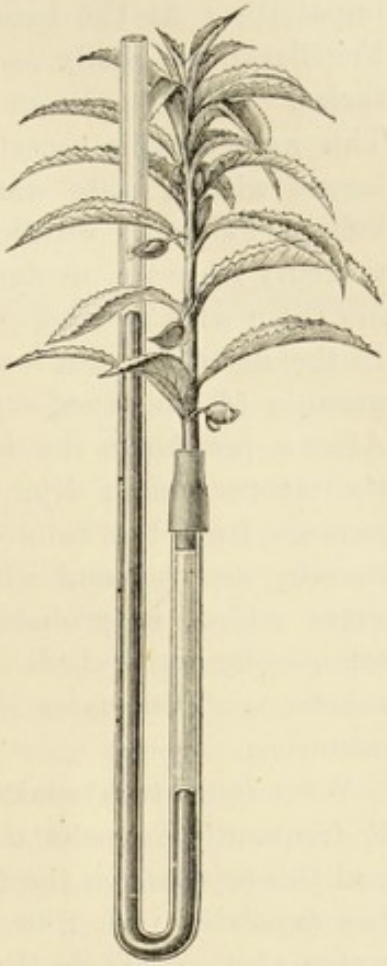

Fig. 78.—Apparatus for investigating the influence of pressure on the escape of water from plant structures.

According to Pitra's account, it should be easy to make the observation that leaf-bearing shoots, cut off, and almost completely immersed upside-down in water, yield fluid in considerable quantities from the cut surface of the stem. I have repeated many of Pitra's experiments, but with negative results. My experience, however, does not extend far enough for a critical examination of the experiments of this author, and therefore I shall not here consider them further. On the other hand, it is possible to prove without difficulty with pieces of the stem of Zea, or Sorghum vulgare, that not only in the roots, but even in the cells of stem structures, pressures may be set up osmotically, which result in escape of fluid. The pieces of stem, about 10 cm. in length, are taken from vigorous maize plants, and from plants of Sorghum which are beginning to flower, the upper section being made at a point a few millimetres above a node. On immersing them in water with the upper cut surface outside the fluid, and covering with a bell-glass, sap soon escapes from the cut surface. If we dry the surface with blotting-paper, it soon becomes moist again.

But other causes besides osmotic pressure may give rise to an

escape of fluid from plant structures. This is the case, *e.g.*, in many nectaries,[2] and we select for examination those of Fritillaria imperialis. At the base of the perianth leaves in the flower of Fritillaria, are easily seen comparatively large bowl-shaped nectaries. There is present in them a sugary sap, containing glucose. This we can easily ascertain by rinsing the base of a few perianth leaves with a little water, and pouring the fluid obtained into boiling Fehling's solution. We further very carefully and repeatedly rinse the nectaries of several perianth leaves with water, dry them with blotting-paper, and then put a bell-glass over them. In the nectaries of a few of the perianth leaves we now place a granule of moistened sugar, reserving the others for comparison. After a few hours the former again contain a sugary sap, while the latter remain dry. The sugar attracts water to itself, by osmosis, from the cells of the nectary, without pressures being thereby set up; and what is effected in the experiment by the sugar added, is probably effected under normal conditions by osmotically active bodies produced by the metamorphosis of the substance of the outer membranes of the epidermal cells of the nectaries.

When felled tree trunks, rich in sap, are exposed to the sun's rays, it frequently happens that fluid exudes from their cut surfaces, and this arises from the fact that the air within the wood elements has expanded owing to rise of temperature, and driven out the water also present in the structures. The following experiments give us further information. Pieces 20–25 cm. long and 3–5 cm. thick are cut in cold but damp weather, in winter, from boughs of willow, elm, ash, hazel or Pavia rubra, which last I chiefly used in my experiments, and, after their ends have been very carefully smoothed, are laid for about twenty-four hours in water at a temperature of about 2° C., so as to increase the quantity of water within them. We then put them in a cylinder containing water at 25–30° C., with their upper ends projecting slightly above the surface. A fairly large quantity of water soon collects on the smooth cut surface on the top of each. I also observed a similar escape of water, resulting from expansion of the air present in the wood elements, when pieces 15 cm. long and 2 cm. thick, cut in winter from branches of Abies pectinata, were placed in water at a temperature of 24° C. When they were subsequently placed in water at 5° C., the water which had issued from the cut sur-

face at the higher temperature was sucked back again owing to the cooling and consequent contraction of the contained air.[3]

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[1] See Moll, *Botan. Zeitung*, 1880.

[2] See Wilson, in *Untersuchungen aus d. botan. Institut zu Tübingen*, Bd. 1, Heft 1.

[3] See Sachs, *Botan. Zeitung*, 1860.

81. The Organisation of Plant Structures and Transpiration.

The tissues of plant structures are by no means all easily permeable to fluid water or to water vapour. On the contrary, there are tissues which allow the passage of water only with extreme difficulty, and to this class belongs especially cork tissue. This fact is of considerable biological interest. Thus we find that many watery structures, *e.g.* potatoes, which have to survive a long period of dormancy, are clothed with a more or less thick layer of cork, and in order to determine its significance, in the maintenance of the watery condition of the parenchyma of the tubers, it is sufficient to make the following experiment.[1] We select two potatoes as nearly as possible of the same size. One of them is peeled in order to remove the cork tissue, the other is left unpeeled. We now weigh the two potatoes, and then place them side by side. Further weighings made at the end of three, six, and twenty-four hours respectively, indicate that the peeled potato loses much more water than its neighbour. The small amount of water which the unpeeled potato loses escapes chiefly from the lenticels and from fine rents in the skin.

If we select two apples as nearly as possible of the same size, peel one of them, leaving the other unpeeled, and determine from time to time, *e.g.* every twenty-four hours, the weight of each, it appears that the apple deprived of its skin gives up far more water to the air than the unpeeled one. Cuticularised epidermis is therefore, like cork tissue, at any rate only very slightly permeable to water.[2]

The small quantity of water given off by unpeeled potatoes and apples is chiefly to be accounted for by the presence of lenticels, and in order to prove directly that these organs are not without importance in determining the amount of transpiration, we make the following experiments. Two pieces of Æsculus or Ampelopsis twigs as similar as may be, without leaves, covered with periderm,

and about 3 gr. in weight, are made air-tight at both ends with sealing-wax, and after being accurately weighed, are laid aside for twenty-four hours. We then again weigh them, and so ascertain the loss by transpiration. We now seal up by means of melted wax, in the one piece the lenticels, in the other patches of periderm of corresponding size, at once weigh both, and then again weigh them after twenty-four hours' transpiration. The piece whose lenticels were sealed will have lost absolutely, or at any rate per cent., less water than the other piece used for comparison.

The epidermis of leaves, as has already been shown in 70, is very generally provided with a layer of wax, which, as is well known, appears in various forms. This coating of wax reduces not inconsiderably the rate of transpiration in leaves, as the following experiment teaches. We may use Eucalyptus globulus, taking the two leaves of the same pair. One leaf is accurately weighed at once, the other after the layer of wax has been wiped off with a soft cloth. Weighings repeated at the end of six, twelve, or twenty-fours respectively indicate that the former leaf undergoes less loss by transpiration than the latter.[3]

The experiments we have made of course allow no doubt to remain that in the transpiration of plants only a small amount at most of the water disappearing in the form of vapour traverses the cuticularised epidermis, especially if the cuticle is richly impregnated with waxy substances. Nevertheless, the cuticle is not completely impervious to water, as the following experiments teach.

We cut off an uninjured leaf of a Begonia, the upper surface of which is devoid of stomata, and lay it together with an object-glass in a crystallising dish. We now treat a large quantity of salt with a little water, so that the salt is slightly moistened, scatter a small quantity of the salt over the upper surface of the leaf, and also on the slide, and then cover the dish with a glass plate. The salt on the leaf rapidly deliquesces, since it attracts water out of the tissue of the leaf; this water can only pass to the outside through the cuticle. The salt on the slide attracts at most a small quantity of aqueous vapour from the atmospheric air, and remains comparatively dry.

We prepare a very readily liquefied mixture by melting together wax and olive oil or cacao butter (a mixture of 1 part of wax and 3 parts of cacao butter is very good). Two leaflets as similar as

possible are cut from a Mahonia; in one leaflet we paint only the upper surface with the mixture, while the other leaflet is only painted on its under surface. In both cases we smear the cut end of the leaf-stalk. After the layer of wax has completely cooled and stiffened, we weigh the leaves, then expose them to the sunlight with their free surfaces directed upwards, and after some time once more weigh them. The leaflet with its under surface untouched will have given off more water in the form of vapour than the other, since the under side of the leaf of Mahonia possesses stomata. The upper side of the Mahonia leaf, which has no stomata, naturally gives off only a comparatively small quantity of water to the air, and this water must traverse the cuticle. Other plants (Ilex, Nerium, Begonia, Ficus, etc.), whose leaves possess stomata only on the under surface, may also be employed for experiments like those with Mahonia.

In investigating the relations between the organisation of plant structures on the one hand, and their transpiration on the other, it must be mentioned that the leaves of many plants, and indeed other organs also, possess tissues which serve for storing up water. Here we have to do especially with plants which vegetate in comparatively dry situations, and which frequently have to endure a long period of great dryness. We may mention particularly many of the Cacti, the Cactus-like Euphorbias, many Crassulaceæ, and species of Aloe and Peperomia. We prepare, *e.g.*, a transverse section of the leaf of Aloe soccotrina. Beneath the very strongly cuticularised epidermis lies the green assimilatory tissue, the cells of which contain comparatively large chlorophyll grains. The middle of the leaf is occupied by a tissue, the so-called water-tissue, the large cells of which are very rich in water, and contain a large quantity of mucilaginous substances. Along the boundary between the water tissue and the assimilatory tissue which surrounds it, lie the vascular bundles. When Aloes, in the dry season, are unable to take up large quantities of water from the soil by means of their roots, use is made of the water stored up in the water tissue, and they do not materially suffer from a drought, which would destroy other plants. The leaf of Peperomia trichocarpa similarly possesses a well-developed water tissue, which is immediately recognised on examination of transverse sections. Under the epidermis of the upper side of the leaf lies a succulent tissue devoid of chlorophyll, the cells of which increase in size towards the middle of the leaf. The assimilatory

parenchyma presents itself in transverse section as a tissue of only slight thickness. It extends between the water-tissue of the upper side of the leaf and a well-developed layer of parenchyma on the under side of the leaf, whose cells certainly contain some chlorophyll, but may likewise, from the quantity of sap which they contain, be regarded as mainly of importance for the storage of water.

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[1] See Detmer, *Journal f. Landwirthschaft*, 1879, p. 119.
[2] See Just in Cohn's *Beiträge zur Biologie der Pflanzen*, 1875, Heft 3.
[3] See G. Haberlandt, *Physiologische Pflanzenanatomie*, 1884, p. 69.

82. Further Experiments Respecting Transpiration.

It is noteworthy that considerably less water evaporates in unit time from a given area of leaf surface than from an equal area of water. To prove this I made the following experiment, using the apparatus drawn in Fig. 79:—A glass vessel, 11 cm. high and 8 cm. wide, is filled with good garden soil, in which a bean seed is placed to germinate. When the young plant has completely unfolded its primordial leaves, the vessel, whose rim has been smeared with fat, is covered with a glass plate divided into halves and provided with three holes. Through the middle hole passes the stem of the plant, packed, if necessary, with cotton wool. One of the two lateral holes receives a thermometer, while the other is fitted with a cork. We now weigh the whole apparatus described, and also a crystallising glass containing water. This last in an experiment of mine was 5 cm. in diameter. After twenty-four hours we again weigh the apparatus. The plant in my ex-

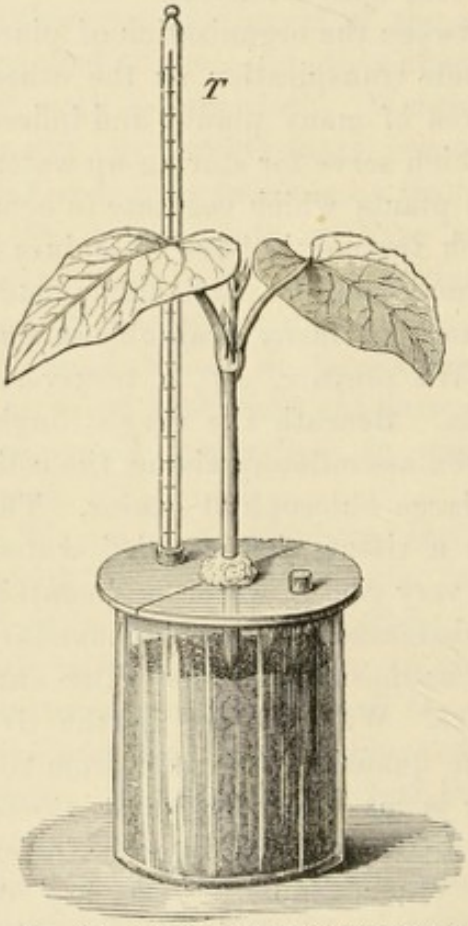

FIG. 79.—Apparatus for transpiration experiments.

periment gave off to the air 4·6 gr., the free water surface 2·23 gr. of water. The area of water exposed was 19·6 sq. cm. The surface of the bean leaves is estimated as follows:—Very homogeneous paper is soaked with a solution of Potassium bichromate, and after drying we determine the weight of a piece of the paper of known area. The two primordial leaves of the bean plant are cut off and placed on sufficiently large pieces of the prepared paper, which are then exposed for some time to direct sunlight. The outline of the leaves is soon clearly recognisable on the paper, since the uncovered parts become very brown in colour. We now carefully cut out the portions of the paper corresponding with the leaves, and determine their weight. Given this, and the weight of a piece of paper of known area, we can at once estimate the area of the leaves. I found the total area of the two primordial leaves of my plant to be 230·8 sq. cm. This area of leaf certainly evaporated not more than 4·6 gr. of water, since I have quite neglected the surface of the leaf-stalks of the stem and of the terminal buds, which, of course, also give off small quantities of water to the air. 19·6 sq. cm. of water surface gave off in twenty-four hours 2·23 gr. of water, which is equivalent to 11·3 gr. per 100 sq. cm. 230·8 sq. cm. of leaf surface lost 4·6 gr. of water in twenty-four hours; therefore 100 sq. cm. would lose 1·99 gr.

It has already been proved in 81, that the cuticularised regions of the epidermis of leaves have a certain significance in connection with transpiration, since, as we have seen, they are not completely impervious to aqueous vapour. But still it is certain that the greatest importance in this respect belongs to the stomata. It is possible, that is to say, to demonstrate a decided connection between the amount of transpiration of a plant organ and the number of stomata present. A direct proportionality between the rate of transpiration and the number of stomata present on a particular area of leaf is not found, it is true; but we need not wonder at this, since the aqueous vapour escaping from the stomata is formed, of course, in the intercellular spaces, and the amount of transpiration depends therefore, not only on the number of stomata, but also on the form, size, and number of the intercellular spaces. Garreau[1] has determined the amount of transpiration on the upper and lower sides of numerous leaves, and at the same time the number of stomata present on equal areas of leaf surface. He obtained the following results:—

	Relative number of stomata.	Quantity of water (expressed in grams) transpired in twenty-four hours.
Atropa belladonna.	above 10	0·48
	below 55	0·60
Nicotiana rustica	above 15	0·57
	below 20	0·80
Tilia europæa	above 0	0·20
	below 60	0·49

In order to repeat Garreau's investigations, we fit up the apparatus represented in Fig. 80. Two similar bell-glasses, of different size according to requirements (about 40–80 mm. in diameter, and 100 mm. in height), are applied to the upper and under side of the same leaf, and cemented air-tight. I have found a mixture prepared by melting together 2 parts of olive oil, 1 part of mutton suet, and 1 part of wax, very serviceable as a cement. If the temperature in the neighbourhood of the apparatus is relatively high, *e.g.* over 20° C., a smaller proportion of olive oil must be used. The bell-glasses must be tubulated, so that we can attach to them the two oil manometers, m and m'. Lastly, within each bell-glass is placed a tube filled with Calcium chloride, g, g'. The increase in weight of these tubes indicates the amount of transpiration from the leaf surfaces. It is obvious that we must experiment not with cut-off leaves, but with leaves remaining intact on the plants. In an investigation conducted under my supervision with Begonia, using bell-glasses 42 mm. in diameter, the following results were obtained:—In four hours, at 20° C., 0·0075 gr. of water evaporated from the upper side of the leaf, while the under side gave off 0·0520 gr.

Stahl[2] has recently made us acquainted with a method of research (Cobalt test), which serves excellently for many experiments on transpiration, and may also be employed to show that the cuticular transpiration falls far behind the stomatal transpiration. Swedish filter paper is impregnated with a 4–5 per cent. aqueous solution of Cobaltous chloride, and dried in an oven or in the sun. We still further dry a piece of the Cobalt paper over a spirit flame or gas flame so that it appears intensely blue, and then lay it on a dry sheet of glass. On the paper we place a leaf of Phaseolus multiflorus, and cover this with a second piece of the dried Cobalt paper and a second sheet of glass. If the leaf was perfectly fresh, and had been exposed to the sunlight before

use, we see, even after a very short time (a few seconds to a minute), that the paper in contact with the lower surface of the leaf assumes a red tint, while the other retains its blue colour. This result is always obtained when we experiment with leaves (leaves of Phaseolus, Salix Caprea, Populus nigra, Liriodendron, Syringa vulgaris, Cyclamen—leaves of the last plant are available even in winter) whose upper surface contains few or no stomata. The reddening of the paper is due to the action on the Cobaltous chloride of the aqueous vapour escaping from the stomata; only traces at most traverse the cuticle, so that the paper in contact with the upper leaf surface suffers little or no change of colour.

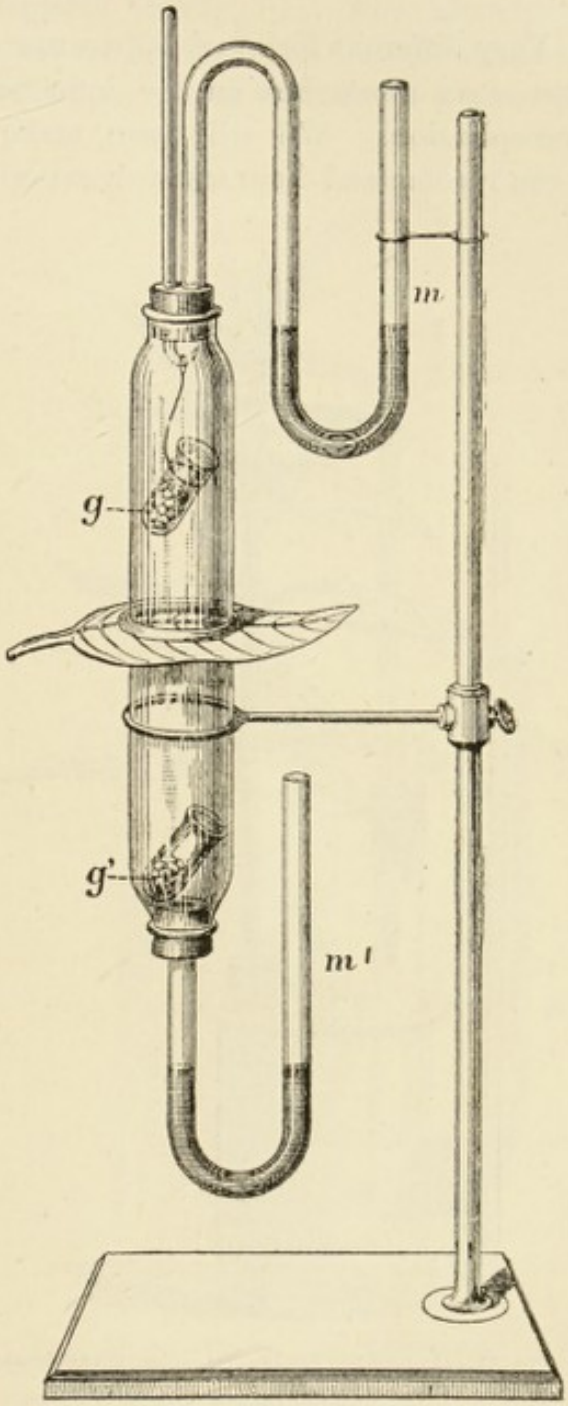

FIG. 80.—Apparatus for investigating transpiration.

In investigating the evaporation of a leaf on the intact plant, it is generally advisable to employ large thin sheets of mica instead of the heavy glass plates. They may be fixed by means of small clamps.

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[1] See Garreau, *Ann. d. Sc. Nat.*, 1850.
[2] See Stahl, *Botan. Zeitung.*, 1894.

83. The Influence of External Conditions on the Transpiration of Plants.

Very different forms of apparatus have been employed in the numerous researches on the influence of external conditions on transpiration. We will here make use of only a few which I can recommend from my own experience. One has already been described in 82 and represented in Fig. 79. It is there indicated that the plants may be cultivated in the covered glass vessel itself. We may also grow them in flower-pots, and place these in a sufficiently large glass vessel with a ground rim and provided with a glass cover. If we are working with comparatively large plants (*e.g.* Helianthus, Nicotiana), and therefore have to use large flower-pots in order to ensure very vigorous growth, we place the pots in receivers made of sheet zinc, and provided with divided covers. In each cover there must be a hole to receive a thermometer, and another for the stem of the plant to pass through. The junction of the halves of the cover may be made air-tight by smearing with a cement (1 part of wax and $\frac{1}{2}$ part olive oil melted together). Experiments with comparatively large plants (*e.g.* Helianthus, Nicotiana) are instructive because they show us that even in a short time, *e.g.* twenty-four hours, they give off to the air a very considerable quantity of water.

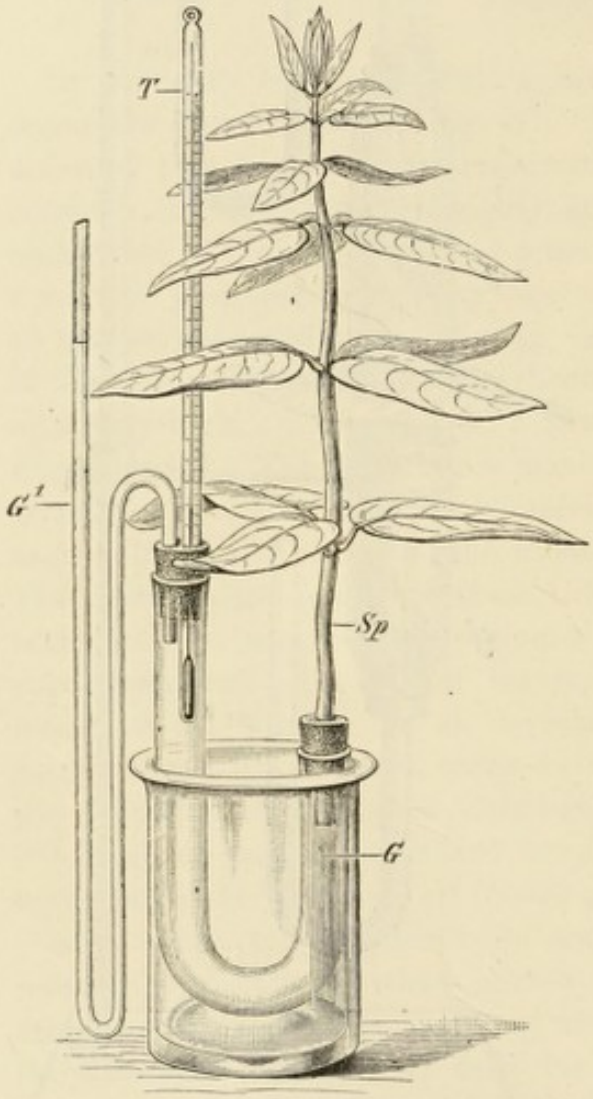

Fig. 81.—Apparatus for investigating transpiration.

The second arrangement which may be conveniently employed in experiments on the evaporation from plants, especially in lecture demonstrations, is depicted in Fig. 81. The U-shaped, fairly wide glass tube *G* is filled with water. One limb is closed by a cork, through which passes the lower end of the shoot, *Sp*, whose transpiration is to be measured. The other limb is closed by a two-holed cork. Through one hole passes the thermometer *T*, through the other one limb of the narrow bent glass tube *G'*, which is filled with water. We place the arrangement in a wide glass vessel, and transfer the whole apparatus to a balance. We are now in a position to determine the loss of weight suffered by the shoot as the result of transpiration. We can, however, at the same time observe the loss of water directly, since the evaporation causes a depression of the column of water in the tube *G'*.

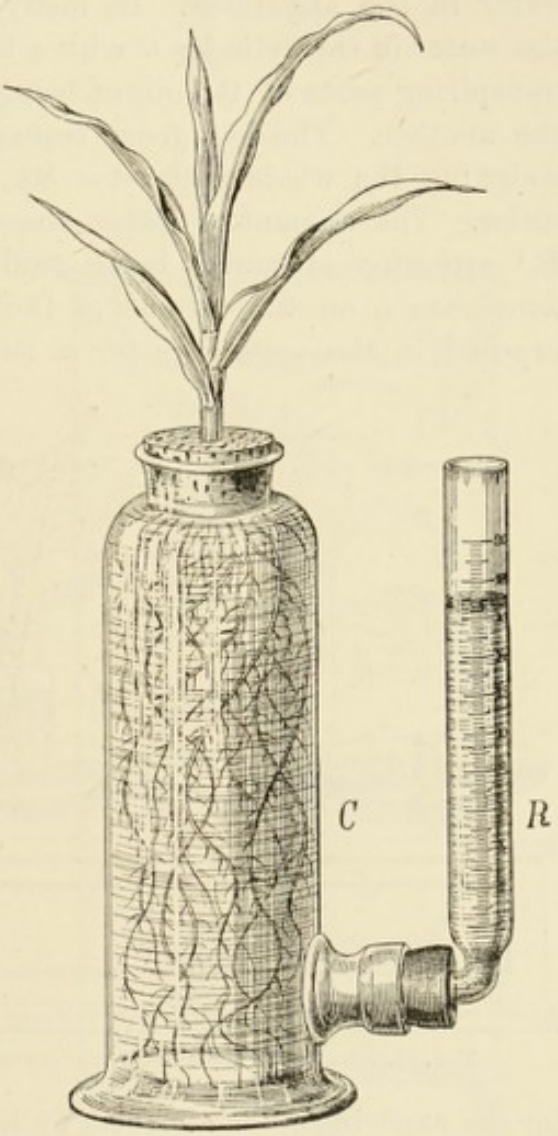

FIG. 82.—Apparatus for determining the amount of water absorbed and given off by transpiring plants. (After Pfeffer.)

The apparatus indicated in Fig. 82 is especially to be recommended for investigating transpiration. The object under examination is fixed water-tight into the upper end of the vessel *C*, which contains water, and is, say, 25 cm. in height and about 6 cm. in diameter. We may experiment either with shoots or with rooted plants. In the former case we close the vessel with a perforated rubber stopper, and pass the shoot through it; in the latter we support the plant by means of a divided cork, the halves of this being

cemented together with a mixture of wax, mutton fat, and olive oil, which melts at a comparatively low temperature. Into the tubulus towards the bottom of the cylinder *C* is fitted the graduated glass tube *R*, about 15 mm. in diameter, and containing water. The water in *R* is covered with a thin layer of oil. It is well to arrange for determining the temperature of the water in the apparatus. In many cases it is advisable to cover the water in the cylinder *C* with a layer of oil, the absorbing and transpiring parts of the object being thus simply separated from one another. The loss from transpiration can be estimated by weighing the whole apparatus on a sufficiently sensitive pair of scales. The amount of water absorbed is indicated by the tube *R*,[1] attention of course being paid to the effect of changes of temperature on the volume of the water. If a rooted plant be exposed in the apparatus for a long time to constant external influences, equilibrium is set up between transpiration and absorption of water. But if, owing to increased temperature of the air, the transpiration is much intensified, the plant loses more water than it absorbs; if, however, it is now covered with a bell-glass, the absorption of water preponderates.[2] Good material for the experiments is afforded by bean or maize plants developed by the water-culture method, and cut shoots of woody plants, *e.g.* Salix. In many cases, *e.g.* for purposes of demonstration, it is sufficient to place cut shoots in a vessel of water, and cover the water with a layer of olive oil. The loss from transpiration is ascertained by weighing the apparatus. If very great accuracy is not required, we may use scales (see Fig. 83). We may work with twigs of Salix fragilis, Helianthus, Eucalyptus globulus (a young shoot of this plant provided with twenty leaves transpired

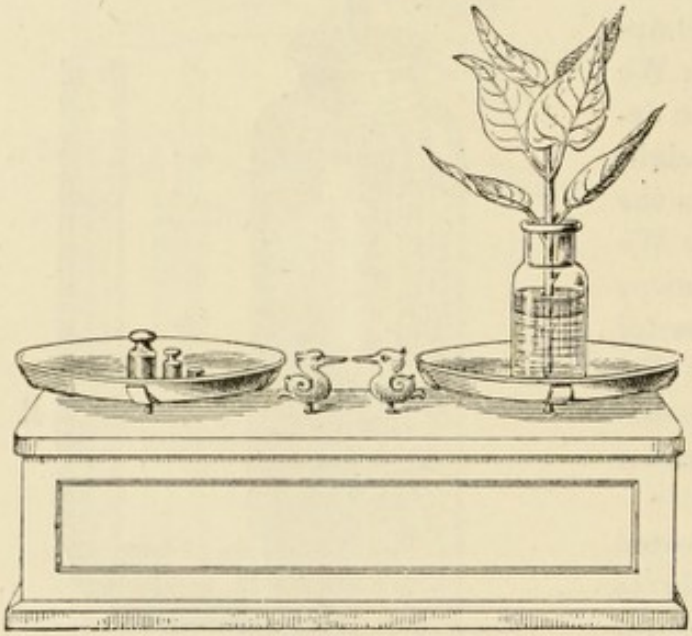

Fig. 83.—Scales for transpiration experiments.

under by no means very favourable conditions 7 gr. of water per hour), etc. In winter we employ for this and other transpiration experiments shoots of Fuchsia, which, especially in direct sunlight, transpire very strongly.

To make sure that plants transpire far more feebly in a moist atmosphere than in one poor in aqueous vapour, we first place our apparatus for half an hour under a bell-glass whose inner surface has been moistened with water. We then expose it for half an hour to the air. We make the experiment, however, not in the open air, but in a room, taking care to keep the plant exposed to the same conditions of temperature and illumination throughout the experiment.

At a high temperature a plant gives off much more vapour than at a low one. The temperature in itself and the hygrometric conditions usually co-operate in bringing this about.

To bring out very clearly the dependence of the amount of transpiration on the quantity of water in the air and on the temperature, I made experiments with the apparatus illustrated in Fig. 84. On a tripod stands the zinc cylinder *Z*, 40 cm. high and 24 cm. in diameter. In the cylinder is a porcelain dish, *P*, resting on a small tripod, while the

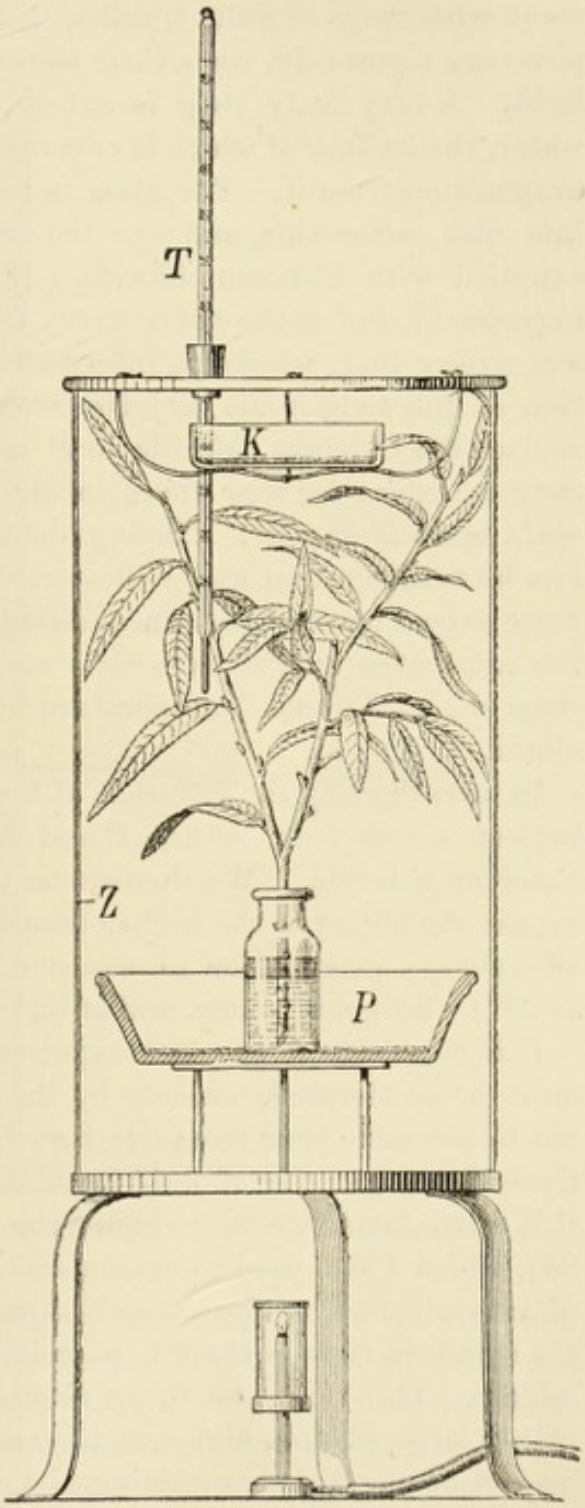

FIG. 84.—Apparatus for investigating transpiration. The zinc cylinder Z is supposed to be transparent.

crystallising glass *K* is supported by an arrangement of wire, which is attached to the under side of the wooden, or better metallic, cover closing the cylinder. The temperature of the air within the cylinder is indicated by the thermometer *T*. We may experiment with twigs of Salix fragilis, which even in the dark transpire very vigorously, since their stomata do not close in absence of light. A very leafy twig is cut off, put into a glass containing water, the surface of which is covered with a layer of oil to prevent evaporation from it. The glass is now placed in the large porcelain dish, after this, and also the crystallising glass *K*, has been supplied with Calcium chloride. If at the commencement of the experiment, and at the end of every four hours, we weigh the glass and willow twig, we obtain information as to the rate of transpiration of the twig in air very free from aqueous vapour. We now replace the Calcium chloride in *P* and *K* by water, and let the twig transpire for four hours in air which is now well supplied with aqueous vapour. Having determined the relatively slight loss by transpiration under these circumstances, the twig is once more exposed to air poor in aqueous vapour. It is best to place the apparatus in a room with a north aspect, because in such a room the variations of temperature in the course of a day are only slight.

In investigating the influence of temperature, the twigs are first kept at a low temperature, *P* and *K* having been charged with Calcium chloride. We then warm the air in the apparatus, and expose the object to the higher temperature. A willow twig gave off (always in air poor in aqueous vapour) in every four hours at 21° C. 5·2 gr. of water, and at 32° C. 8·5 gr. of water.

It is clear that in the last experiment the rate of transpiration must be accelerated, not only by the high temperature of the air, but at the same time from the fact that the object absorbs a relatively large quantity of fluid from the warm water. To exclude this latter factor we may employ the following apparatus (see Fig. 85), which I also used to demonstrate the dependence of the rate of transpiration on the amount of water present. On the ring of the stand *St* rests a shallow porcelain dish, *P*, perforated in the middle. The bell-glass *G*, provided a thermometer, *T*, should be rather large (30 cm. high and 15 cm. in diameter). Through the cork *K* of the water-containing U-tube, *U*, pass the thermometer *T* and the base of a shoot (*e.g.* Syringa), the leaves of the shoot being covered by the bell-glass *G*. The shoot is fixed in the hole

of the dish by means of cotton wool. The cork K' receives one limb of the glass tube Gl, which is about 2 mm. in calibre. The other limb of this tube runs alongside a millimetre scale.

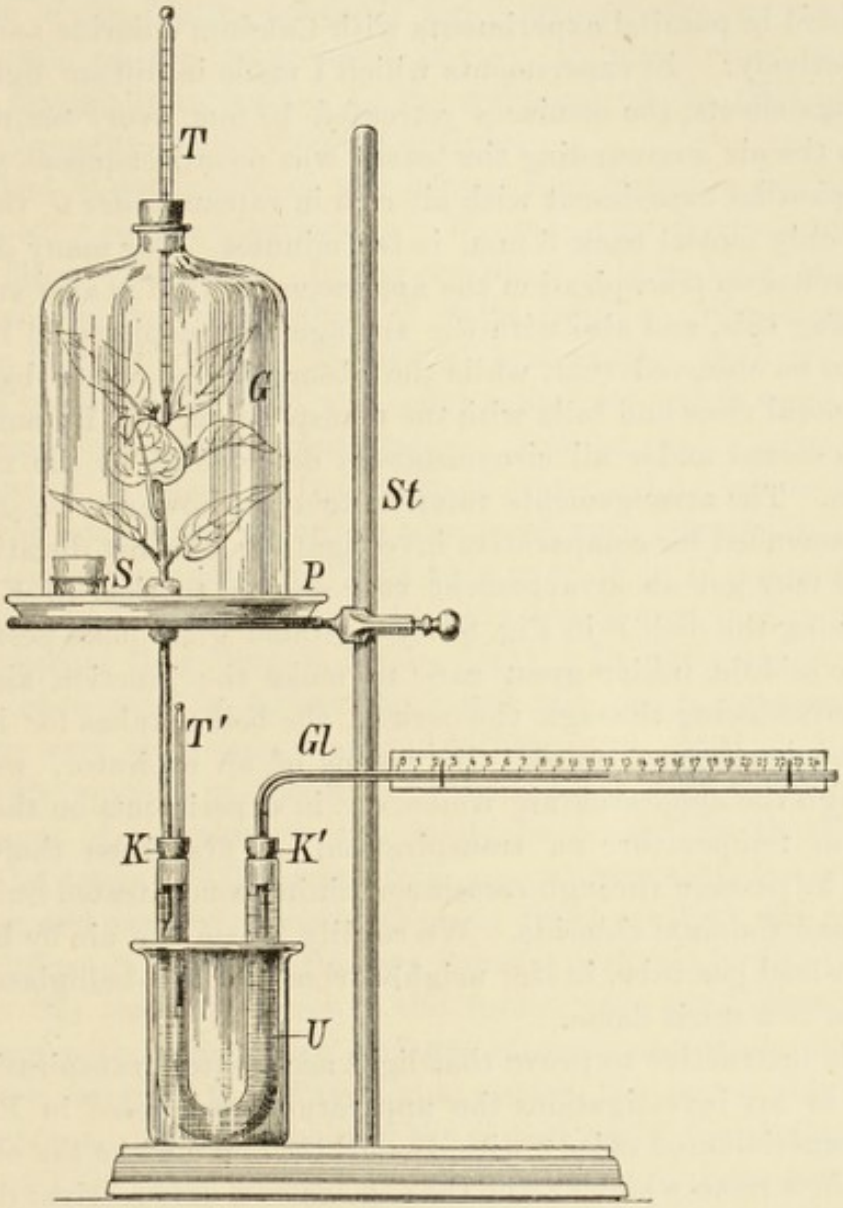

FIG. 85.—Apparatus for measuring the absorption of water by transpiring plants.

At the commencement of the experiment U and Gl are completely filled with water, the bottom of P is covered with chloride of Calcium, and the bell-glass is put on. After a time we ascertain the position of the meniscus in Gl, and can now determine the consumption of water by the plant by noting, say every five minutes, how far the meniscus in Gl is carried back. To raise the temperature of the air under G by a few degrees C., it is only

necessary to introduce into the apparatus the small dish *S* containing hot sand. It may be supported on a small tripod. In observations on the influence of the quantity of aqueous vapour present in the air on the consumption of water by the plants, *P* is provided in parallel experiments with Calcium chloride and water respectively. In experiments which I made in diffuse light with Syringa shoots, the meniscus retreated 10 mm. every ten minutes when the air surrounding the leaves was poor in aqueous vapour. In a parallel experiment with air rich in vapour under *G*, the meniscus only moved back 3 mm. in ten minutes. For many detailed researches on transpiration the apparatus of Kohl is also suitable. In using this, and also with the arrangement depicted in Fig. 85, it is to be observed that, while the absorption of water by plants in general rises and falls with the transpiration, still its amount is by no means under all circumstances determined by the transpiration. The arrangements referred to may, however, be strongly recommended for comparative investigations of short duration.

We may get an arrangement very similar to that of Kohl by replacing the dish *P* in Fig. 85 by a ground glass plate perforated in the middle, taking great care to make the junction air-tight, and introducing through the cork of the bell *G* tubes for leading in and leading off gases. By means of an aspirator, we suck through the apparatus air which, *e.g.* in experiments on the influence of temperature on transpiration, has first been thoroughly dried by passage through vessels containing concentrated Sulphuric acid and Calcium chloride. We readily warm the air by heating the in-lead gas tube, in the neighbourhood of the bell-glass *G*, by means of a spirit flame.

It is instructive to prove that light accelerates transpiration. I used in my investigations the apparatus represented in Fig. 79, and experimented chiefly with Cucurbita. We make the observations in a room which during the experiments only receives diffused light, and can without difficulty be quickly darkened, absolute exclusion of light, however, not being at all necessary. It is best to place the apparatus in front of a window on a balance. We illuminate it for one or two hours, then darken, *e.g.* by closing the window shutters, and again illuminate at the end of one or two hours, and so on. During each period the temperature of the soil and air must be kept constant, and, further, the hygrometric conditions of the air must not vary. As a hygrometer, it is convenient to use a simple stand supporting two thermometers, of which one

is kept dry, while the bulb of the other is wrapped with wet linen. The results of my investigations are detailed in my *Beiträge zur Theorie des Wurzeldrucks*, Jena, 1877, p. 77. The cause of the increase in transpiration under illumination is to be sought on the one hand in the heating effect of light rays penetrating into the plant, while on the other hand it may, in many cases, be still further materially intensified by the widening of the slits of the stomata brought about by access of light.

This last factor is also of the utmost importance in explaining the following simple experiments. We take two narrow-mouthed bottles filled with water. In one we place a large twig of Tilia grandifolia, in the other a shoot of Salix fragilis. We cover the water in both with a layer of olive oil, and weigh. Both are then exposed to diffuse daylight, and after a time we weigh again, and so determine the loss by transpiration. I found that a large Tilia shoot lost in $1\frac{1}{2}$ hours only 0·5 gr. of water, while willow twigs under the conditions described transpired very vigorously. Exposed to direct sunlight, the Tilia twig gave off in $1\frac{1}{2}$ hours 45 gr. of water. In Tilia the stomata are only widely open in direct sunlight, whereas they close in diffuse light. In Salix the stomata remain open even in diffuse light, and the shoots consequently transpire very vigorously even under these conditions.

That the stomata of the lime do really react very energetically to changes of illumination, can easily be proved by the Cobalt test. Cut twigs of Tilia grandifolia are put with their lower ends in water, and exposed, some of them to direct sunlight, the rest to feeble diffuse daylight. After two hours it is shown by the Cobalt test (see 82) that the leaves of the former turn the Cobalt paper in contact with their under surfaces strongly red, while those of the latter, exposed to diffuse light, redden the paper only to a slight extent. With the help of the Cobalt test I found also that leaves of Tilia twigs, exposed to direct sunlight without access of water, rapidly closed their stomata.

The stomata of Aspidistra elatior and Ficus elastica, according to Stahl's observations, also react unusually energetically to changes of illumination. In direct sunlight they open their stomata widely, while in not too bright diffuse daylight, even at a high temperature (30° C.), the stomata remain closed. Aspidistra and Ficus consequently transpire very feebly in diffuse daylight, very energetically in direct sunlight. If a pot plant of Ficus elastica, which has been sunned for about an hour, is weighed,

and then again weighed after exposure for two to four hours to direct sunlight, a considerable loss from transpiration is observed. If now the plant is placed at the back of a room with a north aspect, and we begin a new experiment after the plant has been exposed for about an hour to the changed conditions, then the weighings indicate very slight losses by transpiration. In these experiments loss of water from the pot and soil may be prevented by covering the soil with tinfoil, and wrapping the pot with a white cloth, or, for greater safety, we may proceed as in the experiment illustrated by Fig. 81.

We cut off twigs of Salix fragilis and Tilia grandifolia. The twigs, which must be approximately of the same weight, are brought on to a table in a room with a north aspect. They are not supplied with water. Repeated weighings during one to two days show that the willow twig transpires much more strongly than the lime twig. Therefore also the leaves of the willow are already dry when the lime leaves still contain a comparatively large quantity of water. If we examine withering leaves of lime and willow (leaves of, *e.g.*, Cyperus alternifolius behave quite like the latter) by the Cobalt test, we find the stomata of the lime closed and those of the willow open. The lime and many other plants are able to regulate the intensity of their transpiration. They close their stomata when they are threatened with danger of withering. Willows cannot do this; their leaves consequently dry very rapidly when supply of water is prevented.

To make experiments in winter which teach that withering leaves close their stomata, we employ species of Tradescantia from the hothouse. Freshly cut leaves redden Cobalt paper brought against their lower surface, while slightly withered leaves can no longer do this. If shoots of Cyperus alternifolius and leaves of Aspidistra, which are also always available in winter, are cut and left in diffuse light, without supply of water, the former rapidly wither because their stomata cannot close, the latter for a long time keep fresh since their stomata are shut. The Cobalt test may also be conveniently used here to gain information as to the state of the stomata.

Specimens of Cucurbita or other plants are investigated as to their transpiration by means of the apparatus represented in Fig. 79, or that in Fig. 82. They are first left standing on a balance for half an hour at rest, and the loss by transpiration is determined. Then they are violently shaken for a few seconds, and it

is at once found that a disproportionately large quantity of aqueous vapour has escaped during that time. Sudden shaking increases considerably the transpiration of plants. I have often satisfied myself of this.[3]

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[1] See Pfeffer, *Handbuch der Pflanzenphysiologie*, Bd. 1, pp. 135 and 141.

[2] On the relation between absorption and loss of water in plants, see especially Vesque, *Ann. d. Sc. Nat.*, 1876 and 1878.

[3] Literature: Unger, *Anatomie u. Physiologie d. Pflanzen*, 1855; Sachs, *Handbuch d. Experimentalphysiologie d. Pflanzen*, 1865; Baranetzky, *Botan. Zeitung*, 1872; Wiesner, *Sitzungsber. d. Akadem. d. Wiss. in Wien*, 1876, Octoberheft; Detmer, *Beiträge zur Theorie d. Wurzeldrucks*, Jena, 1877, p. 47; Kohl, *Transpiration d. Pflanzen*, Braunschweig, 1886; Eberdt, *Transpiration d. Pflanzen*, Marburg, 1889.

84. The Wood as a Tissue for the Conduction of Water, and the Influence of Transpiration on the Movement of Water in Plants.

From the base of a very leafy branch of a tree or shrub (I experimented with Pavia rubra), without separating it from the parent plant, we remove a ring of cortex about 5 cm. broad, and extending inwards as far as the wood.

The shoot remains fresh for a considerable time, notwithstanding the fact that the leaves are actively transpiring, since conduction of water is not prevented by the ringing. Thus the bark cannot be regarded as a tissue possessing any considerable importance in connection with the conduction of water along the stem; it is in the wood of the fibrovascular bundles that the water travels. The dry or even partly destroyed pith in the middle of woody stem-structures naturally plays no part in the movement of water in the plant.

Moreover, it is certain that not the whole of the wood conducts water, but only the splint wood, the heart wood having become incapable of conduction. Naturally only plants in which there is a well-marked differentiation between splint and heart wood are suitable for experiments on this subject. If, *e.g.*, we select in summer a Robinia trunk about 12–16 cm. in diameter, and saw through it all round to the heart wood, the plant withers often on the same day. If from a vigorous shoot of Rhus typhina we remove a ring of bark a few centimetres wide, the shoot rapidly

withers. In Rhus typhina the splint is so thin that the ringing injures it, and the conduction of water is necessarily interrupted.[1] In my experiments with Rhus typhina the part above the ring was withered even after a few hours. Rhus glabra does not behave in the same manner.

We cut off a shoot of Impatiens noli-me-tangere, or I. parviflora, and place it with its cut surface in an aqueous solution of methyl green. The stalks of these plants are very transparent, so that we can observe in a remarkably beautiful manner the phenomena here under consideration. In an experiment with Impatiens parviflora it was found that the colouring matter had already risen in the stem of a fairly actively transpiring shoot to a height of a few centimetres at the end of a quarter of an hour, and microscopic examination of transverse sections of the stem showed that only the wood of the vascular bundles, which are arranged in a circle, was stained.

The results of this and similar experiments have recently acquired great significance from the researches of Wieler[2] and Strasburger.[3] They may indeed be utilised to establish the position that the movement of water in the plant takes place in the wood. In discussing, however, the results of these experiments with pigment solutions, it is always necessary, especially as regards details, to be very cautious.

A leaf-bearing branch of Robinia, about 20 mm. in diameter, which has been standing for some time in water, is placed in an aqueous solution of eosin, without its cut surface being exposed to the air. The eosin solution must be so dilute that a thickness of 10 cm. is still transparent. Examination at the end of a few hours teaches that only the outer parts of the wood, not the central parts, are stained. The latter no longer conduct water. If the experiment does not continue too long, it is also easy to prove, from the distribution of the eosin, that only the tracheal paths, and not wood fibres, take part in the conduction of the water. The latter remain unstained.

If Tilia branches are treated in the same way, and examined after a few hours, the eosin can only be detected in the vessels and tracheides. Wood fibres and bast remain unstained. In experiments with Aristolochia the wood stains; the ring of sclerenchyma fibres remains unstained.

Having thus determined that the movement of the water takes place in the tracheal channels, we will now show that the conduc-

tion does not, as was supposed, take place in the lignified membranes of the elements of the xylem, but in their cavities.[4]

I made investigations on this subject as follows:—Two shoots of Salix fragilis (*a* and *b*) are cut and placed with their bases in glasses of water. The water for *a* was covered with a layer of olive oil: *a* and *b* were now placed in the apparatus depicted in Fig. 84, the porcelain dish and crystallising glass having previously been supplied with Calcium chloride, and the air in the zinc cylinder having been heated to 32° C. After an hour *b* was taken out and put in a glass containing 3 per cent. gelatine solution, which was then covered with olive oil. *a* and *b* were now kept in the apparatus for another hour. The shoots transpired, and *b* in doing so absorbed the gelatine solution. At the end of the time both of them were taken out of the apparatus. A piece a few cm. in length was cut from the base of *b*, and the shoot was dipped with the fresh-cut surface in water; both were now left at 20° C. The gelatine stiffened in the vessels of *b*, and this interfered with the movement of water in them. Thus at the end of twenty-four hours the leaves of *b* were withered, while those of the control shoot *a* still appeared fresh.

The following experiment leads to the same result.[5] At midday, or in the afternoon of a sunny summer day, shoots of Vitis are cut under a gelatine solution (20 parts of gelatine to 100 parts of water) tinted with Indian ink. The fluid is heated to 33° C., at which temperature it is perfectly fluid. If we now rapidly put the shoots with their bases in cold water, and renew the cut surface, they soon wither, since the wood elements cannot conduct water owing to the plugging of their lumina by the congealed gelatine. Shoots of Vitis, cut off under water and left standing in it, keep fresh for a long time. (See also Strasburger's cited work, p. 697.)

As regards the forces by which the raising of water in the plant is brought about, these are not yet determined with certainty. Unfortunately there is still no theory as to the movement of water in plants which is satisfactory from all sides.[6] Perhaps the views advanced by Westermeyer and Godlewski as to the causes of the water current come nearest to the truth; Strasburger's view, however, may perhaps be correct.* We must trust to the future

* I have not yet been able to repeat Strasburger's remarkable experiments with killed plants.

to bring deeper insight, and this is not the place to pursue the subject further. We will therefore proceed to study various phenomena (see 85 also as to the ease with which water moves in the wood), which are at all events of great importance in helping us to realise the causes of the movement of water in plants.

We cut off two shoots of Impatiens as similar as possible, and place them both with their lower ends in water. Both shoots are first left for a few hours under a large bell-glass, the wall of which has been moistened on the inside with water. We then put them with their cut ends in a solution of methyl green or eosin. One shoot is exposed to conditions which permit vigorous transpiration. The other we protect as far as possible from loss of water by leaving it under a bell-glass. In the latter the pigment solution rises only slightly, while in the actively transpiring shoot it rises in a short time to a considerable height in the vascular bundles.

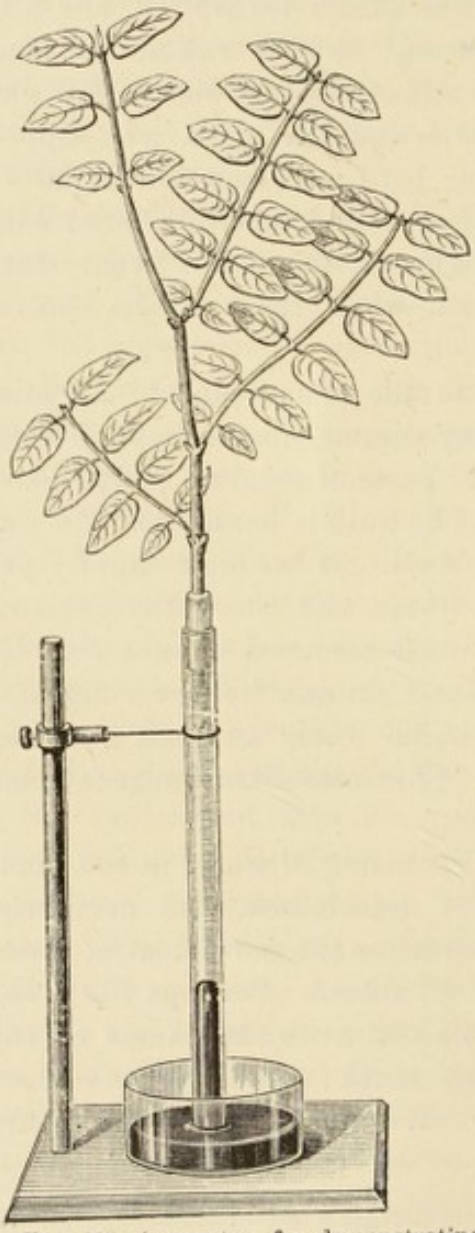

Fig. 86.—Apparatus for demonstrating suction due to transpiration.

The effect of transpiration may also be determined by experiments of another kind. We employ for the purpose the apparatus drawn in Fig. 86. By means of a piece of rubber tubing a leafy shoot is connected air-tight with a straight glass tube, which is then filled with water and dipped into mercury. As the water is used up in consequence of transpiration, mercury enters the tube. I found that during fairly active transpiration it may rise several centimetres in a few hours (I used, *e.g.*, shoots of Lonicera tatarica).

If in the early spring (at the end of March or the beginning of

April) we bore a hole into the trunk of a birch tree, not far above the ground, and by means of rubber tubing or sealing wax fasten into it, air-tight, one limb of a glass tube bent at right angles, it will be found, especially during the night, that a considerable quantity of fluid will be forced out of the tree owing to root pressure. If we repeat the experiment in summer, *e.g.* in June, not a drop of sap flows out, and indeed water may even be sucked up into the stem, as may easily be determined by dipping the free end of the tube into water. This phenomenon is the result of transpiration.

If a transpiring plant, the elements of whose wood at first contain large quantities of water, has not at its disposal a good supply of fluid—and this will especially be the case in summer—then the water gradually disappears from the lumina of the wood vessels and tracheides.

As was shown earlier, air only passes with difficulty from the intercellular air system into the cellular system, and the quantity of air which the water present in plants holds in solution, and under certain conditions can give off to its surroundings, is not considerable. Therefore, during strong transpiration in summer, the wood elements do not contain water alone, but in addition there is present a large quantity of moist rarefied air, a fact which has already been referred to on p. 182.

If we cut out a piece of wood from a transpiring tree, and throw it into water, it will float on the surface. If the lumina of the wood elements had been completely filled with water, it would have sunk, since the specific gravity of wood substance in itself is greater than that of water. A piece of fresh wood, however, thrown into water gradually sinks deeper in the fluid, and at the same time it increases in weight, a fact which can only be due to absorption of water.

The following investigation also teaches that while under some conditions the lumina of the wood elements are completely filled with water, this can certainly not be the case always.[7] Two vigorous specimens of Cucurbita, or of some species of Begonia, are decapitated and provided with tubes in the manner shown in Fig. 73, one of the plants having previously been allowed to transpire for a day, while the other has been protected from considerable loss of water by covering with a bell-glass. The latter plant at once yields sap, which under the influence of root pressure rises in the tube. The former at first does not yield sap; it even

somewhat eagerly sucks up water poured into the tube. The sap only gradually begins to flow.

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[1] Dutrochet, *Mémoires pour servir à l'histoire anatomique et physiologique de végétaux*, 1837, p. 193.

[2] See Wieler, *Jahrbücher f. wissenschl. Botanik*, Bd. 19.

[3] See Strasburger, *Bau und Verrichtung der Leitungsbahnen*, Jena, 1891, pp. 549 and 569.

[4] See Elfving, *Botan. Zeitung*, 1882, p. 714; Vesque, *Ann. d. sc. nat. Bot.*, VI. Sér., T. XIX., p. 188; Scheit, *Botan. Zeitung*, 1884, p. 201; Errera, *Comptes rend. de la soc. roy. de bot. de Belgique*, T. XXV., II. Th., p. 28; Strasburger, *Bau und Verrichtung der Leitungsbahnen*, Jena, 1891, p. 541.

[5] See Errera, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 4, p. 16.

[6] Literature respecting the movement of water: Sachs, *Lectures on the Physiology of Plants*; Hartig, *Gasdrucktheorie*, 1883; Westermeyer, *Berichte d. Deutschen botan. Gesellschaft*, 1883; Godlewski, *Jahrbücher f. wissenschl. Botanik*, Bd. 15; Scheit, *Jenaische Zeitschrift f. Naturwissenschaft*, N.F. Bd. 12; Strasburger, *Bau und Verrichtung der Leitungsbahnen in den Pflanzen*, Jena, 1891; Schwendener, *Sitzungsberichte d. Akadem. d. Wiss. zu Berlin*, 1892.

[7] See Detmer, *Beiträge zur Theorie d. Wurzeldrucks*, in Preyer's *Physiologische Abhandlungen*, 1877, Bd. 1, Heft 8, p. 37.

85. The Mobility of the Water in the Wood.

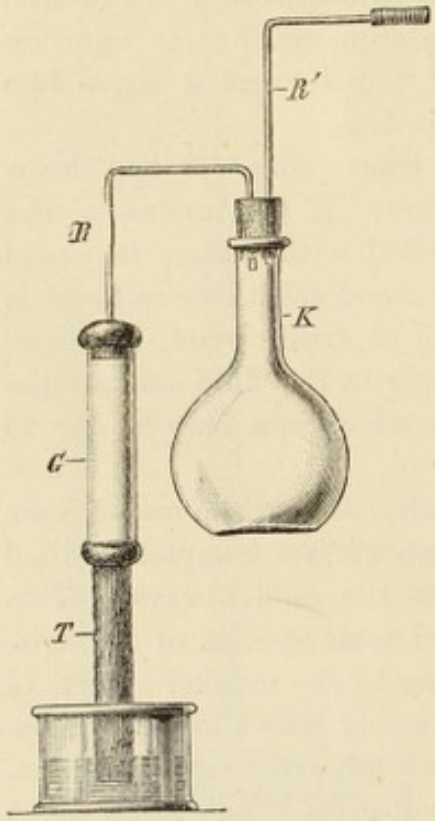

FIG. 87.—Apparatus for demonstrating the readiness with which water moves in the wood.

We cut from a trunk or bough of Abies pectinata, 2–4 cm. in diameter, a piece 15–30 cm. in length. It must be very rich in water, and therefore it is best to take it in winter from a living plant, and possibly place it in water for some time before the experiment. The carefully smoothed cut surfaces of the wood appear dry. If, however, holding the object vertically, we place a thin layer of water on its upper surface with a paint brush, we may observe that this water rapidly sinks into the wood, while the lower cut surface becomes moist. Rapidly turning the piece of wood upside down, a repetition of the process is to be observed. A minimal pressure is therefore sufficient to set in motion the

threads of water present in the wood. The following experiment leads to the same conclusion. If we fix a piece of fresh fir wood in the shorter limb of a U-shaped glass tube, and fill the tube with water, fluid escapes from the upper cut surface until the pressure is completely equalised. A straight glass tube, *G* (see Fig. 87), 2–4 cm. wide, is closed at one end with a perforated rubber stopper, through which passes the thin glass tube *R*. Into the other end is cemented air-tight a fresh piece of fir-wood (I used a piece 15 cm. long and 2 cm. in diameter). The tube *R*, bent twice at right angles, is in connection with the flask *K*, which in turn is connected by means of the glass tube *R'* with an air-pump. If we dip the free cut surface of the wood into water and exhaust, water at once filters through the wood into the wide glass tube. We now put together the apparatus represented in Fig. 88.

The very large funnel *T* hangs in the iron ring of a heavy ring stand placed on a high cupboard. The tube of the funnel is connected by rubber tubing with the glass tube *G*, about 150 cm. in length, whose lower end passes into a wide glass tube, *G'*. The lower part of this last is closed air-tight with a piece of a branch of Abies pectinata or Taxus baccata, *A*, 6 cm. long and 2 cm. in diameter. The whole apparatus is now filled with dis-

FIG. 88.—Apparatus for demonstrating the readiness with which water moves in the wood, and the fact that the closing membranes of the bordered pits of the tracheïdes are imperforate.

tilled water free from dust. The pressure forces the water through the piece of branch, which may be either used with or without removal of the cortex, so that in a short time a considerable quantity of water filters through. The rate of flow becomes less in time as we readily observe. This happens even if we take care to keep the level of the water in the funnel always at the same height, and the cause of the phenomenon is to be sought in a gradual change (*Verunreinigung*) in the cut surface of the wood through which the water enters.

We now make another experiment with our apparatus, filling it not with clean water but with water containing very finely divided Cinnabar in suspension. A large quantity of distilled water is treated with the best Cinnabar, and the fluid is filtered several times, so that only the very finest particles remain suspended in it, which do not settle to the bottom even in the course of several days. The water which filters in the course of one to two days through the cylinder of wood at the lower end of our apparatus is perfectly clear. Examination of the piece of wood itself shows that only its upper end, to a depth of a few millimetres, is impregnated with Cinnabar. Microscopic examination of delicate sections of the wood demonstrates the presence of Cinnabar in the tracheides, and we ultimately come to the following interpretation of the results of our experiment.

Naturally the tracheides at the cut surface of the cylinder of wood used in the experiment have been opened in cutting it. During filtration, water and Cinnabar penetrate into these tracheides. No doubt at all now exists that water, even under a minimal pressure, filters through the closing membranes of the bordered pits of tracheides; all our experiments bring this fact clearly into view. The particles of Cinnabar, however, are not able to pass from one tracheide into another, since they cannot traverse the closing membranes of the pits. At the same time the experiment offers direct proof of the presence of closing membranes between the elements of coniferous wood.[1]

The ease with which water filters through wood is of great importance in relation to the passage of water in the wood masses of plants.

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[1] See Th. Hartig, *Botan. Zeitung*, 1863, and especially Sachs, *Arbeiten d. botan. Instituts in Würzburg*, Bd. 2, p. 296.

86. The Rate at which Water Moves in the Plant.

It has often been attempted to form an idea of the rapidity with which water moves in the plant by placing the objects of investigation with their bases in a solution of colouring matter, and determining the height at which the colouring matter can be detected after a certain time. This method, however, cannot lead to accurate results. The absorbed pigment solution, viz., disintegrates. The colouring matter is arrested by certain elements of the tissue (especially the lignified ones), while the water travels on. We can easily convince ourselves that such an analysis of the pigment solutions is possible by pouring into a tall glass cylinder an aqueous solution of methyl green or eosin (the latter of such strength that a layer 10 cm. in thickness is still transparent), and covering with a glass plate to which a narrow strip of blotting-paper has been attached so as just to dip at the bottom into the pigment solution. After a short time the colouring matter will have risen to a certain height in the paper; but the paper is moist beyond the limit to which the pigment has penetrated. The water has thus risen higher than the pigment.

If, on the other hand, we allow a strip of blotting-paper to dip into a solution of Lithium nitrate of about 2 p.c. strength, it is easy to prove that the Lithium salt rises to the same height as the water. Thus, if we cut off the uppermost portion of the paper reached by the fluid, and hold it in the flame of a Bunsen burner, the presence of Lithium can be readily observed spectroscopically (by the presence of the well-known red Lithium line in the spectrum). The solution of Lithium nitrate has often been used by Sachs[1] for determining the rate of movement of water in plants, and we will make our experiments according to his method.

It is best at first, for reasons which are not far to seek, to experiment with perfectly intact rooted plants, and not with portions severed from the parent. We may, *e.g.*, employ willows. Shoots of last year are cut in spring, and placed in food solutions. In a few months, when the shoots have developed a copious root system and numerous leaves, they are ready for investigation. Maize plants raised by the water-culture method may also be employed; and so also may pot plants of Nicotiana, Cucurbita, and Helianthus, etc., grown in good garden soil. The plants must be vigorous and very leafy. One or two days before the investigation proper begins, we place them in front of a window with a south

aspect, so that they are exposed to the sunshine and high temperature. During this period the soil in the pots is not watered. In experiments on the absorption of Lithium solution, plants vegetating in food solutions must be removed from them immediately before the experiments begin, and placed in a 2 per cent. solution of Lithium nitrate. If, on the contrary, we work with pot plants, the soil in the pots must be thoroughly soaked with a 2 per cent. solution of Lithium nitrate. The plants are now left exposed to very favourable conditions for transpiration. At the end of an hour we sever each of the stems above the soil, cut it up from above downwards into small pieces, and cut off the leaves. In these operations great cleanliness is necessary in order to avoid conveying the Lithium salt which may be present in one part of the plant, by means of the knife, to another part. To test for Lithium, thin pieces of the stem or small pieces of leaf are taken by the forceps and held in a glass flame, towards which the spectroscope is directed. Larger quantities of Lithium can be recognised immediately, smaller quantities only when the ash glows. To avoid obtaining too high a value for the height to which the Lithium salt (and therefore also the water) ascends, we must always reckon the distance of the highest part of the stem or highest leaf in which Lithium is detected, only from the root-neck [collum]. It is also frequently a good plan not to decapitate the plant, but simply to cut off pieces of leaf after the experiment has proceeded for a certain time, and test these for Lithium. It is worthy of observation that we can sometimes recognise considerable quantities of absorbed Lithium in the leaves of the plants when it is not to be detected in the lower parts of the stem, a fact of which I satisfied myself in testing Sachs' method for determinating the rate of water conduction in plants. Often the Lithium obviously accumulates in the leaf tissues in larger quantities than in the stem. Sachs made the following determinations of the height to which water and Lithium salts ascend in plants.

	Ascent per hour.
Salix fragilis	85 cm.
Zea Mais	36 „
Nicotiana Tabacum	118 „
Cucurbita Pepo	63 „
Helianthus annuus	63 „

Recently experiments with pigment solutions for determining

the rate of water conduction have gained importance, because Strasburger[2] has shown that in particular eosin solutions of the concentration already specified serve very well for the experiments even with separated plant structures. The results obtained with this pigment are, it is true, not absolutely exact, but still they merit attention. On a hot summer day, a shoot of a plant of Humulus or Bryonia growing in the open is cut through at the base under water. The cut surface is now left for half an hour or an hour in the water, and the shoot is then conveyed to the eosin solution. This precaution is necessary to prevent the pigment solution being forced into the object by the atmospheric pressure. The eosin rises very rapidly in the transpiring shoot. It may, *e.g.*, advance 2–3 m. in half an hour, as is shown by examination of transverse sections.

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[1] Sachs, *Arbeiten des botan. Instituts in Würzburg*, Bd. 2, p. 148.
[2] See Strasburger, *Histologische Beiträge*, Heft III., pp. 550 and 590.

87. The Withering of Plants.

When a plant constantly loses by transpiration more water than it gains by absorption, it gradually withers. Its leaves hang down limp, and if water is not supplied to it, it ultimately dries up. If, however, we water the soil in flower-pots in which just withered plants (*e.g.* beans or vegetable marrows) are growing, the cells of the leaves quickly become turgescent again, and the plants rapidly assume once more a fresh appearance. The same thing is seen if instead of supplying water to the plants we reduce their transpiration, *e.g.* by placing them under a bell-glass.

If very leafy shoots of trees or shrubs are cut off and placed with the lower end of their woody stems in water, they usually remain fresh for days. It is therefore surprising that in some plants the shoots, when so treated, wither very rapidly, in spite of the fact that their stems are very woody. According to my observations branches of Salix fragilis often behave in this manner. In general, however, shoots cut off and placed in water, remain fresh for a longer time the further the development of the wood in the stem has advanced. If we cut off, for example, shoots of Helianthus tuberosus, about 1 metre in length, and place them in water, they keep fresh throughout for several days; shoots 20–30 cm. long, on the other hand, very quickly wither

when placed in water, the younger unfolded leaves becoming limp first, and then the older ones.

We now make the following very instructive experiment with Helianthus tuberosus. We bend down a long shoot without separating it from the plant, and without cracking it, so that a portion 20 cm. from the summit dips into the water contained in a vessel placed below it, the summit of the stem, and the leaves themselves, not being wetted. We now with a sharp knife cut through the stem below the water, so as to sever from the parent a length of 20 cm. at the end of the shoot, taking special care that the cut surface is not exposed to the air at all, but remains throughout below the surface of the water. Our shoot keeps fresh for days, while other Helianthus shoots of the same length (20 cm.) cut off in the air, and then at once (say after one to two minutes) placed in water, rapidly wither. We may, however, in various ways render them turgescent again. If we cut off a few of the withered leaves, those left quickly become fresh again, since the losses of the shoot by transpiration are now covered by the absorption of water. A withered Helianthus shoot is fixed air-tight by means of rubber tubing or a rubber stopper in the shorter limb of a U-shaped glass tube containing water, so that its cut end dips into the water. Mercury is now poured into the longer limb of the tube. A pressure of a few cm. of mercury is not indeed sufficient to revive the withered shoot, but if the water is forced into the withered shoot with a mercurial pressure of 30–50 cm., it becomes turgescent again. With a sharp knife we cut under water a length of 5 cm. from the base of a withered Helianthus shoot, 20 cm. long, standing in water, taking care that the new cut surface does not come into contact with the air. The shoot will quickly assume a fresh turgescent appearance.

Our experiments with Helianthus shoots, which we may repeat with other plants, teach first of all the important fact that the shoots wither when cut off in air and then placed in water, while they keep fresh when cut off under water. There are several reasons for this. When a portion of the plant is cut off in the air, mucilaginous or gummy substances exuding from the surface of the wound do not get removed. They are left adhering to the cut surface, and so render the tissue less capable of absorbing water. Moreover, the negative pressure which occurs in the elements of the wood of uninjured transpiring plants (see p. 182) is more or less neutralised when the shoot is severed in the air, and

the conduction of water in the stem is thereby greatly prejudiced. These various injurious results do not appear when the shoot is cut through under water, as it is easy to see.[1]

[1] See H. de Vries in *Arbeiten d. botan. Instituts in Würzburg*, Bd. 1, p. 287, and F. v. Höhnel in Haberlandt's *Wissensch.-prakt. Forschungen auf d. Gebiete d. Pflanzenbaues*, Bd. 2, p. 120.

VII. THE ABSORPTION OF MINERAL SUBSTANCES BY PLANTS.

88. The Roots of Plants as Organs for the Absorption of Mineral Substances.

The roots serve to fix the plant in the soil, but they also function as organs for the absorption of water and mineral substances. If we cultivate plants by the water-culture method in aqueous food solutions, the latter function is exhibited in the clearest manner. But even in the soil in which plants grow, food solutions are present in abundance, for the fluids retained by the elements of the soul, and circulating between them, are not pure water, but dilute food solutions. Water acts upon the elements of the soil, dissolving and disintegrating them. They give up to the water mineral substances, absorbed or still more closely held by them, and the solvent power of the water is frequently greatly increased by the presence of large quantities of Carbon dioxide originating in the soil through processes of decay.

I shall not here enter into details concerning the absorption of mineral substances from food solutions by roots, since this question is to be considered in 89. We shall, however, refer to a few observations which are of interest in connection with the behaviour of roots in the soil.

We germinate a few wheat-grains in good garden soil, in a flower-pot, and carefully remove the young plants from it when they have developed four or five roots. If we vigorously shake the seedlings, a large part of the soil adhering to the roots falls off, but a certain proportion does not loosen itself. The whole surface of the roots is clothed by a layer of soil; only the root-tips are free from clinging particles of soil. Careful microscopical examination of the roots shows that their tips are not provided with root-hairs, while they are very abundantly supplied with

them over the rest of their surface. To these elongated unicellular organs the fine elements of the soil cling very closely; the hairs actually coalesce with the particles of earth, as is easily seen under high magnification. The root-hairs are the organs by means of which the absorption of water and mineral substances is especially effected. They withdraw from the soil the dilute food solutions present in it, but further, by acting in the manner described in 91 on the closely applied elements of the soil, they themselves prepare food solutions for the plants, which at once pass over into the organism.

If we grow plants of Triticum vulgare for about five weeks in good garden earth, and then carefully take them out of the soil, it is found, after vigorous shaking, that no soil remains clinging to the root-tips or to the older parts of the roots, but that the younger regions of the organs behind the growing points do retain it. These younger regions are covered with numerous root-hairs, while the hairs of the older parts of the root have already perished.[1]

Various observers have determined that the appearance of root-hairs on the roots of plants is dependent on a series of external factors, of which moisture must be regarded as the most important.[2] We grow seedlings of Zea, Avena, Triticum, Pisum, Phaseolus, in fairly moist garden soil, and determine by microscopic examination of delicate transverse or longitudinal sections that moderately developed roots have under these conditions produced numerous hairs. We also raise a few plants of the kinds named without putting them into soil, laying the seeds after soaking, or after the commencement of germination, on netting stretched over a beaker of water. We place this in a crystallising glass containing water, and cover it with a bell-glass, whose rim must dip into the water in the glass. During germination we prevent the plants suffering from scarcity of Oxygen by frequently removing the bell-glass. The roots of many plants (Avena, Triticum) thus developing in water, have root-hairs like the roots of plants grown in somewhat moist earth, as I satisfied myself by experiments with Triticum vulgare. According to Fr. Schwarz, the roots of Zea, Pisum, and Phaseolus, on the contrary, do not develop root-hairs when grown in contact with water. I must, however, remark that at least in the case of Zea Mais, I saw numerous root-hairs appear on quite normal and straight main roots which had developed in water. Perhaps the roots of

different kinds of maize do not behave in the same manner in this respect. Perhaps also it is not a matter of indifference whether the roots develop in spring water, or in distilled water, or whether they are grown in darkness or light. I found that the main roots of peas grown in distilled water, in absence of light, were devoid of root-hairs, while roots grown in garden soil were very well supplied with them.

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[1] See Sachs, *Handbuch der Experimentalphysiologie der Pflanzen*, 1865, p. 185.

[2] See Fr. Schwarz in *Untersuchungen aus d. botan. Institut zu Tübingen*, Bd. 1, H. 2.

89. The Absorption of Mineral Substances from Nutrient Solutions by Roots.

In my *Lehrbuch der Pflanzenphysiologie*, p. 136, I have already pointed out that the conditions relating to the absorption of mineral substances by roots from solutions of food stuffs are of a very complicated nature, and are still by no means satisfactorily elucidated. The result which is finally attained is not only dependent on the concentration of the solution, the nature of the food stuffs employed, their consumption in the plant, etc., but also on the nature of the plant, the external conditions under which it is growing, and many other circumstances. Further investigations, with careful analysis of the whole phenomenon, will be required in order to gain fuller information. In this place we will consider the absorption of mineral substance by roots which have at their disposal an aqueous solution of a single salt, a question which is certainly of interest in connection with the absorption of mineral substances from complete food solutions.

We soak a number of well-developed seeds of Phaseolus or Zea, germinate them in moist sawdust, and determine the weight of each seedling. Glass vessels of rather more than 100 c.c. capacity are fitted with perforated corks, and in the holes of these we fix the seedlings singly by means of cotton wool. Some of the vessels have been previously supplied with 100 c.c. of a 0·250 per cent. solution of Potassium nitrate, others with a 100 c.c. of a 0·050 per cent. or 0·025 per cent. solution of that salt. We now weigh the vessels with their seedlings, and then leave them in a well-lighted place until they have lost about 50 gr. in weight, so that about half of the fluid originally present has been sucked up by

the roots. The plants are now removed from the solutions, each is washed with distilled water (which is then added to the residual fluid in the corresponding vessel), dried with blotting-paper, and weighed. We thus ascertain on the one hand the quantity of water which the plants have lost in the form of vapour, and on the other the amount of water which has gone to form a vital part of the plants. There is a source of error in our experiment, due to the fact that the cotton wool does not close the vessels air-tight, and so a small quantity of water can escape into the air without the help of the plants. It is, however, easy to determine the magnitude of the error, and eliminate it from the result by filling with 100 c.c. of salt solution a few glasses not provided with plants, but merely closed with a cork and cotton wool, and then determining the loss of weight which these undergo during the experiment. Similarly there is, of course, no difficulty at all in taking into consideration the increase in dry weight which the plants undergo during the period of vegetation. Finally, we determine the weight of salt contained in the fluid remaining in the glasses, by boiling to dryness and weighing the residue. Thus all the data for calculation are before us. From these we find that bean plants absorb from 0·250 per cent. solutions of Potassium nitrate relatively much water and but little salt, the fluid left in the glasses being therefore more concentrated than the solution originally supplied to the plants (de Saussure's law [1]). In contact with 0·050 per cent. or 0·025 per cent. solutions of Potassium nitrate, on the other hand, the plants absorb a comparatively concentrated solution, the fluid remaining in the vessels being more dilute than the solution originally provided.[2] At all events, then, we have the interesting fact that the roots of plants absorb solutions placed at their disposal not necessarily in the form in which they are supplied, but with a particular quantity of water they take up, according to circumstances, sometimes a smaller, sometimes a larger quantity of salt. In conclusion, we may obtain an approximately correct result if we grow bean seedlings, as above, in glass vessels holding 100 c.c. of Potassium nitrate solutions of different strengths, and without further weighings simply determine the amount of salt in the fluids left in the vessels after half of the original solution has been absorbed.

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[1] See de Saussure, *Recherches sur la végétation*, 1804, p. 247.

[2] See W. Wolf, *Versuchsstationen*, Bd. 6 and 7.

90. Corrosion Phenomena.

Roots are not only able to supply plants with food stuffs by the absorption of ready-made food solutions, but they are also able to withdraw from the compact elements of the soil absorbed, or even still more firmly bound, substances. The absorbing cells of the roots, especially the root-hairs, give out, at all events, as we shall see in 91, certain substances which, on reaching the membranes of the root-hairs, impregnated as these are with water and closely applied to the particles of the soil, must exert a solvent action on the soil particles. In this way the elements of the soil undergo corrosion, and the substances thus dissolved by the agency of the roots themselves pass over into the plant.

To prove that roots can set up corrosive action, we make the following experiment. A small flower-pot is about half filled with moist sand, on which we now place a slab of marble carefully polished on its upper surface (the marble plate which I used, and which in the course of the experiment became corroded in the manner represented in Fig. 89, was 45 mm. in diameter and 7 mm. thick). The flower-pot is now completely filled up with moist sand, and in this is laid a soaked seed of Phaseolus, which quickly begins to germinate. The roots of the plant force their way downwards into the sand, and after some time they encounter the slab of marble. Over this they grow horizontally till they come to the edge of it, when they again grow more or less vertically downwards in the sand. If we stop the experiment, remove the marble from the soil, wash it with water and dry with a soft towel, we shall find on its upper surface an exact representation of the roots which were applied to it. The polish has been removed at the places of contact between marble and roots. I obtained, as indicated in Fig. 89, rather broad lines of corrosion, clearly owing to the corrosive action of the root-hairs springing laterally from the roots. The main root growing downwards

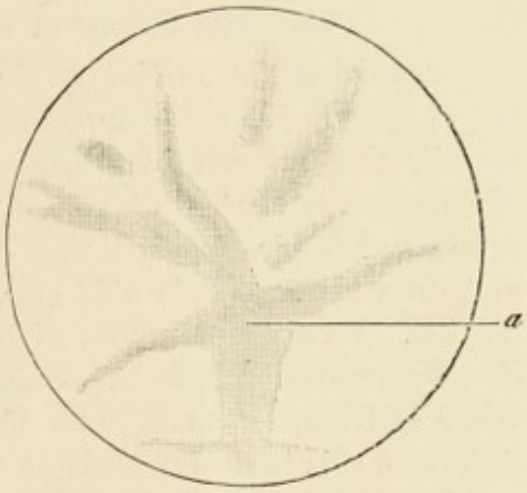

FIG. 89.—Slab of marble, the surface of which has been corroded by the roots of a Phaseolus plant.

touched the marble at *a*; it then took the direction indicated by the broad stripe in the drawing, while the remaining lines of corrosion owe their origin to the lateral roots. It remains to be noted that the bean plants, the corrosive action of whose roots we are investigating, should not be allowed to grow for too long a time (only from ten to fourteen days), since if the experiment lasts long very many roots come into contact with the marble, and the corrosion lines due to the individual roots no longer stand out distinctly.[1]

[1] Corrosion phenomena were first thoroughly studied by Sachs; see his *Handbuch der Experimentalphysiologie der Pflanzen*, 1865, p. 188.

91. The Causes of Corrosion Phenomena.

Corrosive action obviously can only result from the fact that the roots, lying in close approximation to the stones or particles of soil, give out certain substances capable of decomposing them. Naturally the Carbon dioxide, formed in the cells of the root as a product of respiration, will first be thought of; but organic acids, and even Hydrochloric acid also, claim consideration in this connection. If the membranes of the root-cells are permeated with dilute solutions of these substances, then action of the roots on the stony and earthy constituents of the soil is at once rendered possible, and this is clearly brought out by the following experiment.*

We put together the apparatus represented in Fig. 90. The bottle *G* contains dilute Hydrochloric acid. Through the hole of the cork closing this bottle passes the comparatively wide glass tube *R*, covered at its lower end with a piece of pig's bladder. In our apparatus the fluid represents the cell contents, and the bladder the membranes of the root-cells. If we lay a bit of marble on the bladder permeated with dilute Hydrochloric acid, it is soon partially dissolved, and the solution of Calcium chloride formed passes by diffusion into the Hydrochloric acid. We can easily detect the presence of Calcium in the fluid by means of Ammonium oxalate. Similarly the solutions permeating

Fig. 90. — Apparatus for illustrating some features of corrosion phenomena.

* This experiment was first made by Zöller at the suggestion of Liebig.

the membranes of the root-cells act upon the stones and other elements of the soil; corrosive action is set up, and the substances dissolved are absorbed by the plant.

From the following experiments which I recently made, but which need to be carried still further in order to definitely establish the relations in question, it appears to me to follow that not only Carbon dioxide and organic acids, but also Hydrochloric acid, are to be regarded as substances which are of importance in setting up corrosive action. We grow maize plants by the water-culture method (see 1) in a fluid which contains in 1000 gr. of water, 1·00 gr. of Calcium sulphate, 0·25 gr. of Potassium chloride, 0·25 gr. of Magnesium sulphate, 0·25 gr. of acid Potassium phosphate, and a little Ferric chloride. The culture vessels need be large; it is sufficient if they contain 250 c.c. of fluid. When the maize plants have developed their fourth leaf in the non-nitrogenous food solution, they are at any rate in a high state of Nitrogen starvation. Two maize plants are now removed from the food solution, and transferred to two vessels, of which one contains water, *a*, the other a 0·1 per cent. solution of Ammonium chloride, *b*. A third vessel, *c*, is provided with Ammonium chloride solution alone, without a plant; one plant is left in the non-nitrogenous food solution, *d*; another likewise remains in the original food solution, after Ammonium chloride solution (0·1 gr. in 100 c.c. of water) has been added to it, *e*. After about eight days strips of blue litmus paper are dipped for thirty seconds into the fluids in *b* and *e*, others for fifteen seconds into the fluids in *a* and *d*, and then for fifteen seconds into the fluid in *c*. The strips of litmus paper introduced into *b* and *e* become more intensely red in colour than the other two, which can only be explained on the assumption that the Ammonium chloride has been decomposed by the plants with formation of free Hydrochloric acid. The Ammonium chloride penetrating into the tissues of the plants meets with organic acids in the cells. These decompose the chloride, and since the Hydrochloric acid thus formed is not made use of by the plants, it escapes into the food solution, and increases its acidity.

To convince ourselves that organic acids, even outside the plant, can decompose chlorides with formation of Hydrochloric acid, the following experiments may be made:—

We obtain two beakers and pour into each 500 c.c. of distilled water. To *a* we add 3 gr. of Oxalic acid; to *b*, 3 gr. of Oxalic acid and 0·4 gr. of Sodium chloride. We now suspend in each

vessel a slab of marble in the manner described in 25. The fluid in *a* remains clear, owing to the fact that the marble becomes quickly coated with a crust of Calcium oxalate, which retards the action of the Oxalic acid. The fluid in *b*, on the other hand, rapidly becomes very turbid, which can only be explained as due to the following reaction. The Oxalic acid decomposes the Sodium chloride. The marble is acted on by the liberated Hydrochloric acid with formation of Calcium chloride. On this the Oxalic acid now acts, and the Calcium oxalate so formed, which rapidly collects in large quantities at the bottom of the vessel, causes the turbidity of the fluid.

We can satisfy ourselves in still another way that organic acids are able to decompose chlorides.[1] Six culture fluids are prepared: *a*, 15 c.c. of distilled water; *b*, 15 c.c. of water containing 0·020 gr. of Citric acid; *c*, 15 c.c. of water containing 0·7 gr. of Potassium chloride; *d*, 15 c.c. of water containing 0·7 gr. of Sodium chloride; *e*, 15 c.c. of water containing 0·020 gr. of Citric acid, and 0·7 gr. of Potassium chloride; *f*, 15 c.c. of water containing 0·020 gr. of Citric acid, and 0·7 gr. of Sodium chloride. The fluids are now left standing for about twenty-four hours, and then to each we add a few drops of a very dilute aqueous solution of methyl violet. The fluids *a*, *b*, *c*, and *d*, exhibit almost the same violet tint; *e* and *f*, on the other hand, are distinctly blue in colour. This indicates the presence of free Hydrochloric acid, since, while very dilute solutions of Citric acid scarcely affect the colour of methyl violet, it becomes blue in presence of very dilute Hydrochloric acid.*

[1] See Detmer, *Botan. Zeitung*, 1884, No. 50.

92. Absorptive Capacity of the Soil.

It is a highly important fact that the soil is able to retain very energetically (to absorb) a number of substances with which it comes into contact. Potash, ammonia, and Phosphoric acid are most actively absorbed by the soil, and are therefore prevented from sinking deeply into the ground, a fact which is obviously of

* Günsberg's test for Hydrochloric acid in presence of chlorides is also very serviceable here. We mix 1 gr. of vanillin and 2 gr. of phloroglucin with 30 gr. of alcohol. A few drops of this mixture, and a few drops of the fluid under investigation, are placed in a white porcelain dish. If a red colour appears on heating, it indicates the presence of free Hydrochloric acid.

the utmost significance in connection with plant life. If salts soluble in water, and containing potash ammonia or Phosphoric acid, whether they have originated in the soil itself or have been directly added to it from outside, come into contact with the minute particles of the soil, they are absorbed more or less actively, according to the character of the soil. They become chemically fixed, and we will now proceed to determine by suitable experiments the absorption of one substance, viz. ammonia (Knop's method).[1]

100 gr. of air-dry fine earth are intimately mixed with 10 gr. of powdered chalk, and treated in a flask with 200 c.c. of a solution of Ammonium chloride, containing exactly 1 gr. of the salt in 208 c.c. of water. We leave the soil in the fluid for forty-eight hours, frequently shaking it, and then filter off 40 c.c. of fluid, and evaporate this down to about 10 c.c., with addition of a drop of pure Hydrochloric acid. In this 10 c.c. of fluid we determine the quantity of Nitrogen, and also in 40 c.c. of the original Ammonium chloride solution, which has also been similarly concentrated by evaporation to 10 c.c. If the Ammonium chloride solution has been accurately prepared, 40 c.c. of it should contain exactly 40 c.c. of Nitrogen (at 0° C. and 760 mm. barometric pressure). From the results of the Nitrogen determinations, for which we use an Azotometer (see *Zeitschrift f. analytische Chemie*, Bd. 9, p. 226 and Bd. 13, pp. 101 and 383) and brominated soda lye, the quantity of ammonia absorbed by the soil can be easily calculated. The brominated solution (solution of Sodium hypobromite) is prepared by dissolving 100 gr. of caustic soda in 1250 c.c. of water, allowing to cool, and then adding 25 c.c. of Bromine. We use for each experiment 50 c.c. of this fluid to 10 c.c. of the Ammonium chloride solution concentrated by evaporation. To determine the Nitrogen absorbed by 60 c.c. of the generating fluid (50 c.c. of brominated lye and 10 c.c. of water) we employ the table prepared by Dietrich (*Zeitschrift f. analytische Chemie*, Bd. 5). The Azotometer may be obtained from Ehrhardt and Metzger in Darmstadt at a price of about 30 mks.

The apparatus, which is represented in Fig. 91, consists first of a generating vessel divided into two parts by a glass septum—not visible in the figure—which does not extend to the top. In one compartment we place 50 c.c. of the brominated soda, in the other 10 c.c. of the research fluid. The generating vessel is closed with a rubber stopper, and placed in a cooling vessel, which, like the tall

glass cylinder, has been filled with water. Through the cork of the generating vessel passes a glass tube provided with a glass stop-cock. This is connected by means of rubber tubing with the graduated glass tube in the cylinder. The glass stop-cock is pulled out, and the communicating tubes within the cylinder are filled with water by squeezing the rubber ball while keeping the clip open. We now run off the water through the clip till the bottom of the meniscus is accurately at the zero of the graduated tubes. After five minutes the stop-cock is again inserted, but so turned that the generating vessel is left in communication with the graduated tube. We wait for a little time, to see whether the level of the water in the latter changes. If it does, the glass stop-cock must again be taken out, and the water meniscus once more brought to zero.

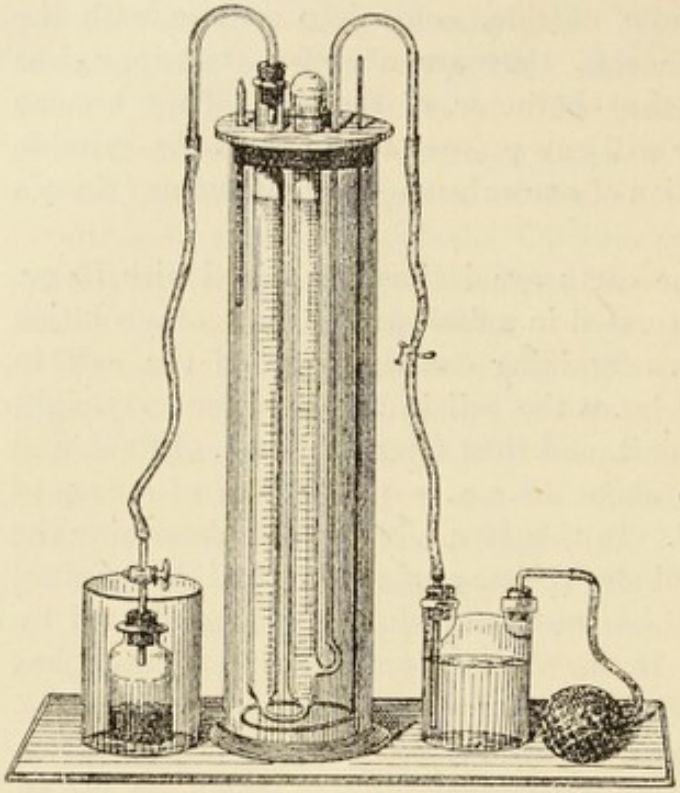

Fig. 91.—Azotometer.

The generating vessel is now removed from the cooling vessel. By tilting it we slowly transfer the brominated soda to the fluid under investigation, after running off through the clip 20–30 c.c. of water. We then close the stop-cock, shake the generating vessel vigorously, and again open the stop-cock so as to allow the liberated Nitrogen to pass over into the graduated glass tube. These operations are repeated several times. Then the generating vessel is returned to the cooling cylinder, and left in communication by means of the stop-cock with the graduated tube. After about fifteen minutes, it will have assumed its original temperature, and we now open the clip, and bring the water to the same level in the two communicating tubes. Finally we read off the

number of cubic centimetres of Nitrogen evolved, the temperature indicated by the thermometer in the cylinder, and the barometric pressure. The reductions of the gas volumes are easily carried out by means of the tables accompanying the apparatus.

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[1] A full account of the absorptive capacity of the soil, and its causes, will be found in Detmer, *Lehrbuch d. Bodenkunde*, Leipsic, 1876.

THIRD SECTION.

Metabolic Processes in the Plant.

I. THE BEHAVIOUR OF NITROGENOUS COMPOUNDS.

93. The Proteids Which can be Isolated from Plant Structures.

We shall here entirely disregard the organised protoplasmic structures of plant-cells, already dealt with in 46, and confine ourselves to showing that various proteids are to be met with in the vegetable organism.

If we grind wheat or barley fruits to powder (about 25 gr.), and leave this in water for some time, we can obtain by filtration a clear liquid in which the presence of albumin may be detected. On heating this fluid the proteid coagulates, and separates out as a clot. If we filter and warm the juice expressed from crushed fruits (*e.g.* grapes), we shall again obtain a coagulum of proteid.

To the second great group of vegetable proteids, the vegetable caseins, belong the legumin of beans, peas, etc., the conglutin of lupins, and the glutin-casein of grasses. We select for somewhat careful investigation conglutin. Seeds of Lupinus luteus are ground up in a small hand mill, the powder (about 25 gr.) is treated with distilled water, and to the mixture is added potash solution until the fluid has a slight alkaline reaction. The remains of the seeds are separated by means of a hair-sieve from the conglutin-containing fluid, which is then filtered and very slightly acidified with Acetic acid. The precipitated conglutin is collected on a filter, and washed with water. Conglutin is insoluble in water. If, however, we suspend some conglutin in water, and treat it with Phosphoric, Acetic or Citric acid, or a solution of Sodium phosphate ($Na_2 H P O_4$), it dissolves.[1]

Representatives of the third group of vegetable proteids are present in specially large quantities in wheat flour. This is mixed with water, and the paste well kneaded between the hands under a continuous fine stream of water. There remains behind a tough elastic mass of glutin with which only small quantities of starch are mixed. Glutin, which is soluble in water containing potash, consists of a series of proteids (glutin-proteids), viz. glutin-fibrin, gliadin, and mucedin, which can be partially isolated from it by means of alcohol.[2]

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[1] For more detailed information see Detmer, in Wollny's *Forschungen auf dem Gebiete der Agriculturphysik*, Bd. 2, Heft 4. I do not here deal with Weyl's researches on vegetable casein (see Detmer, *Lehrbuch der Pflanzenphysiologie*, 1883, p. 157).

[2] For further details consult Ritthausen, *Die Eiweissstoffe der Getreidearten*, 1872, p. 28.

94. Macrochemical and Microchemical Reactions of Proteids.

To familiarise ourselves with one of the more important microchemical proteid reactions (the biuret reaction), an aqueous solution of albumin, or water holding in suspension conglutin from lupin seeds, is heated to boiling, a small quantity of caustic soda solution is added, and into the hot fluid is introduced, by means of a glass rod, a drop of Fehling's solution. The presence of proteids is indicated by a violet coloration of the fluid. To prepare Fehling's solution 34·65 gr. of Copper sulphate, purified by recrystallisation, are dissolved in 200 c.c. of water. Also 173 gr. of Sodium Potassium tartrate are dissolved in 480 c.c. of a solution of caustic soda of sp. gr. 1·14 (about 10 per cent. soda lye). These two solutions are now mixed and diluted up to 1000 c.c. at a temperature of 15° C.

In the cells of the parenchyma of Phaseolus cotyledons there are present, besides starch-grains, large quantities of proteids. We may therefore conveniently employ sections (which must be at least two cells in thickness) of bean cotyledons in order to familiarise ourselves with the microchemical reactions of proteids. We pour into an evaporating dish a few cubic centimetres of concentrated Copper sulphate solution,[1] or a solution of Cupric tartrate,[2] prepared by mixing a solution of 5 parts of Copper sulphate with a solution of 9 parts of normal Potassium tartrate, and filtering off the relatively somewhat insoluble Potas-

sium sulphate produced. The sections are placed in one of the Copper solutions, removed after a few minutes with the forceps, superficially washed by dipping in clean water, and then at once laid in potash solution which is heated to boiling. The contents of the cells take on a violet coloration in virtue of their proteid contents.*

If a section from the cotyledon of a dry pea is mounted on a slide in a drop of glycerine (2 parts of glycerine to 1 part of water), covered with a cover-glass, and treated from the edge of the cover-glass with a drop of Iodine solution, the starch grains at once take on a blue coloration, but the aleurone grains, and the ground mass in which they are embedded, become yellow owing to their richness in proteid.

In contact with sugar and Sulphuric acid proteids become red in colour, and to familiarise ourselves with this reaction we mount sections, *e.g.*, from the cotyledons of a dry bean in a drop of concentrated cane-sugar solution, and run in strong Sulphuric acid from the margin of the cover-glass.

If sections from plant structures rich in proteids are placed on a slide for some minutes in a drop of cold fuming Nitric acid, and then treated with ammonia, they take on an intense yellow colour (Xanthoproteic reaction).

Millon's reagent stains proteids brick-red. It is prepared by treating mercury, without warming, with an equal part by weight of concentrated fuming Nitric acid, and diluting, after the metal has dissolved, with an equal volume of water. It is advisable only to use the reagent when freshly prepared. If we place sections from the cotyledons of Pisum in a drop of the reagent, if necessary slightly warmed, the contents of the cells become disorganised; after a time, however, owing to the presence of proteids they stain brick-red.

In recent years numerous reagents (particularly solvents) have been used for acting on the protoplasmic structures of cells, and on the results obtained has been based the view that a whole series of different proteid substances are present in the protoplasm, cell-nucleus, etc. It is, in fact, certain that, *e.g.*, nuclein, as we shall see in 97, has properties quite different from those of an

* This reaction, however, like others, is not absolutely decisive. The test may also be made by laying the sections on the slide in a drop of Fehling's solution, covering with a cover-glass, and heating till the formation of bubbles ceases.

ordinary proteid. Nevertheless, apart from this and a few other observations, the method referred to, although in principle nothing can be urged against it, has led to no very valuable results. Thus, *e.g.*, from a strict chemical standpoint, we cannot consider most of the results obtained in this direction by Frank Schwartz (see Cohn's *Beiträge zur Biologie d. Pflanzen*, Bd. 5, H. 1) to be of any value.

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[1] See Sachs, Pringsheim's *Jahrbücher*, Bd. 3, p. 187.
[2] See Pfeffer, Pringsheim's *Jahrbücher*, Bd. 8, p. 538.

5. General Considerations Respecting the Behaviour of Proteids in Plants.

The aleurone grains of seeds have already been mentioned in another place. They contain large reserves of proteid, and just as starch grains undergo important changes in the germination of seeds, so also do the aleurone grains undergo changes when germination begins. The protein grains, viz., are dissolved, and their substance is utilised for the formation of living protoplasmic structures. To convince ourselves that such solution does take place, we need only germinate seeds of Ricinus communis, and then examine as thin sections as possible of the endosperm. The proteid grains are no longer seen as glistening structures, as in dormant seeds; their outer part is dissolved up, and mixed with the ground substance to form a cloudy emulsion.

If we grind up lupin seeds in a hand mill, and treat the powder with water, we can readily determine that large quantities of proteid are present in the solution. We need only heat the fluid to boiling, and add some potash and a drop of Fehling's solution. On treating lupin seed powder with water, and also on soaking uninjured seeds, it is conglutin which especially passes into solution. But this proteid is insoluble in pure water. Therefore substances must be present which assist its solution. If we test the reaction of the lupin powder extract by means of litmus paper, it is found to be somewhat strongly acid. This acid reaction is in some cases due to the Citric acid which is present in many kinds of lupin (and conglutin is of course soluble in Citric acid); it may, however, be due to another cause. If we stir up some conglutin in water, the fluid becomes at most only very slightly acid in reaction; but if we now add a solution of Potassium phosphate ($K_2 H P O_4$), which itself has a slight alkaline

reaction, the conglutin dissolves, and the fluid becomes much more strongly acid than before. The proteid removes potash from the $K_2 H P O_4$, and passes into solution, while on the other hand acid Potassium phosphate ($K H_2 P O_4$) is produced. Now the seeds contain, as is known, comparatively large quantities of potash and Phosphoric acid, and if they are exposed to water, then it follows from what has been said that a solution will readily be formed having a strongly acid reaction, and containing large quantities of proteids belonging to the group of vegetable caseins.[1]

Experiments have already been indicated in another place (see 19) teaching that neither ammonia nor free Nitrogen is given off as a result of metabolism during the germination of seeds.

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[1] See Detmer, in Wollny's *Forschungen auf dem Gebiete der Agricultur-physik*, Bd. 2, Heft 4.

96. Pepsin and Peptone.

Proteids as such are not able to pass by osmosis through cell-walls or membranes of a similar character. It is therefore of physiological interest that many plants produce ferments which can convert proteids into peptones, substances which are at least slightly diffusible.

Peptonizing ferments (pepsin) are secreted by the glandular tentacles of Droseras, and are present in the fluid secreted by the pitchers of Nepenthes, and also in many latices[1] (*e.g.* in the latex of Carica papaya). If neither papayotin (which, however, is a commercial article) nor a pepsin-containing latex is available, we may make the following instructive experiment to acquaint ourselves at least with the process of peptonising. A pepsin solution, viz., is easily prepared by extracting fresh pieces of the mucous membrane of a pig's stomach with glycerine, and filtering. If now we heat about 500 c.c. of a 0·2 p.c. aqueous solution of Hydrochloric acid in a porcelain evaporating dish, on the water-bath, to a temperature of 40° C., and digest in it for some time 40 gr. of fibrin, so as to cause the proteid to swell up as much as possible, then the addition of a few drops of the ferment-containing glycerine causes in a few minutes almost complete peptonization and solution of the fibrin. The requisite fibrin (from ox blood) may be obtained from the butcher, and may be preserved in glycerine. To prepare it for experiment, we wash it carefully

with water, and then place it in the warm dilute Hydrochloric acid. If we use the liquid secreted by Nepenthes, or latices, as our pepsin-containing fluids, care must be taken, at least in many cases, that the dilute Hydrochloric acid in which we have placed the fibrin to swell up is kept for a long time at a temperature of 40° C., since in these cases the peptonisation often does not proceed so rapidly. In many cases, however, the presence of pepsin in latices may be detected very quickly, and I found this to be the case, for example, in the following experiment, which can easily be repeated. A few cubic centimetres of very dilute Hydrochloric acid were poured into a test-tube, a few fragments of fibrin were added, and the test-tube was then placed in water at a temperature of 40° C. After the fibrin had swollen up, the fluid was treated with a few drops of latex taken from the stalks of fig fruits cut before ripening. The peptonizing and solution of the fibrin took place in a few moments.

When pepsin acts on proteids, complicated chemical changes take place. Among the ultimate products are various peptones which can easily be detected as such by the biuret reaction. If we warm a small quantity of a peptone-containing fluid, neutralise with potash, and then add Fehling's solution, the mixture does not take on a violet coloration as it does in presence of proteids, but a purple red colour.

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[1] See especially Hansen, *Arbeiten des botan. Instituts in Würzburg*, Bd. 3, Heft 2. Also, respecting the occurrence of pepsin in seedlings, see Neumeister *Zeitschrift f. Biologie*, Bd. 30.

97. Nuclein.

While protoplasm is especially rich in proteids, nuclein must be regarded as a characteristic constituent of the nucleus. The Nitrogen-containing nuclein of the nucleus is distinguished from proteids by containing Phosphorus, and by its characteristic behaviour towards reagents. In the latter connection it is particularly important to notice that nuclein is not attacked by fluids containing pepsin. To observe this we place on the slide a fragment of epidermis from the under side of a Tradescantia leaf (I used with especially good results Tradescantia virginica), and add a drop of pepsin-containing fluid (a mixture of 1 part by volume of glycerine extract of pig's stomach, with 3 parts by volume of

0·2 per cent. Hydrochloric acid). Examination shows that the protoplasm contracts, while the nuclei in the cells rapidly become perfectly homogeneous. The nuclei then increase in volume, and ultimately appear as yellowish, highly refringent structures, which undergo no further change. When the nuclei, after becoming homogeneous, begin to increase in size, the contracted protoplasm at one or more points swells out into a vesicle. Finally this bursts, and now there are left only insignificant protoplasmic residues surrounding the nucleus. Dilute Hydrochloric acid does not alter nuclei treated with the artificial digestive fluid, while they immediately dissolve in dilute soda solution.[1]

[1] See Zacharias, *Botan. Zeitung*, 1881, p. 169.

98. Microscopic Tests for Asparagin.

I have energetically endeavoured to establish the view[1] that the living proteid molecules of the protoplasm, the physiological elements as I term them, under all circumstances, and in every cell in a state of vital activity, break down by dissociation into nitrogenous and non-nitrogenous compounds (dissociation hypothesis). These last are broken down in respiration, and provide the material necessary for the growth of the cells, etc., while the former soon accumulate in greater or less quantities in the cells, or unite with non-nitrogenous substances to reform proteids. Moreover, the nitrogenous products of dissociation of the physiological elements of the protoplasm (asparagin, glutamin, leucin, tyrosin, allantoin) are of great significance, inasmuch as they play an important part in the translocation of substances in the plant.

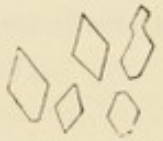

Fig. 92. — Asparagin crystals. (After Zimmermann.)

Of all the nitrogenous decomposition products of proteids, asparagin (amidosuccinamic acid) appears to be of the greatest importance. For this reason we shall give it particular attention, and firstly learn how to detect its presence in plant-cells microchemically.

Asparagin is almost completely insoluble in alcohol, and therefore if we treat a concentrated aqueous solution of it with absolute alcohol, the asparagin separates out. Similarly by means of alcohol we can precipitate asparagin occurring in solution in the

cell-sap, and since the crystals produced (belonging to the rhombic system), especially those which appear in the form of rhombic tables with an obtuse angle of 129° 18′, are of large size and characteristic form, it is possible in this way to demonstrate microchemically the presence of the acid amide in cells [2] (see Fig. 92).

We place the sections (which must be more than one cell thick, so that all the cells are not opened) in a watch-glass containing absolute alcohol, rapidly shake them about in the fluid, and examine them. If only a very small quantity of asparagin is present in the cells, it is best to treat the sections on the slide with absolute alcohol, lay on the cover-glass, and examine the preparation after drying.

To avoid confusing the separated crystals of asparagin with other bodies, *e.g.* crystals of Potassium nitrate, we subsequently treat the sections with a saturated solution of asparagin. If the crystals obtained with alcohol actually consist of asparagin, they will not dissolve. Crystals of other substances are, on the other hand, absorbed by the asparagin solution.

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[1] See Detmer, *Vergleichende Physiologie d. Keimungsprocesses der Samen*, 1880, Pringsheim's *Jahrbücher*, Bd. 12, Wollny's *Forschungen auf d. Gebiete d. Agriculturphysik*, Bd. 5, and *Lehrbuch d. Pflanzenphysiologie*, 1883.

[2] See Pfeffer, Pringsheim's *Jahrbücher*, Bd. 8, p. 533, and Borodin, *Botan. Zeitung*, 1878, p. 804.

99. The Quantitative Determination of the Total Amount of Nitrogen and of the Nitrogen Present in the Proteids and Acid Amides in Seedlings.

From a large quantity of seeds of Lupinus luteus are selected about 300, very normal and alike in development. We determine the average weight of a single seed, and estimate the dry weight of the material (see 1). The seeds are now put to soak for twenty-four hours in water. We then thoroughly moisten some sawdust with water, and fill it into large flower-pots or zinc boxes, rubbing it between the hands, and letting it fall into the pots or zinc boxes so as to form a very loose bed. In this the seeds are laid, covered over with the moist sawdust, and put aside in the dark, at a temperature of say 20° C. It only remains to take care to replace the water lost by evaporation.

After three, five, or seven days we select seedlings which are very similar. They are cleansed, pounded in a mortar, and then dried, at first on the water-bath with frequent stirring, and afterwards in the drying chamber at a temperature of 50° C to 60° C. The residue is now left in the air for twenty-four hours, loosely covered; it is weighed, and then samples are at once taken for determinations of the quantity of dry substance. We now know the dry weight of the seedlings obtained. The dry weight of the corresponding number of seeds is likewise known, and we are therefore in a position to reduce the results of the researches following to quantities of dry substance corresponding with seeds and seedlings respectively.

To determine the total Nitrogen, 1–2 gr. of dry substance of the seeds and seedlings, well powdered, is examined by Kjeldahl's method. (See in König's *Untersuchung landwirthschl. wichtiger Stoffe*, 1891, p. 150.)

The Nitrogen of the proteids is determined by Stutzer's method. (See König, p. 212.)

Subtracting the Nitrogen of the proteids from the total quantity of Nitrogen, we obtain a result giving the quantity of Nitrogen in the non-proteid compounds. The seeds contain only very small quantities of such substances, while the seedlings, especially fairly advanced ones, are rich in amido-acids and acid amides, etc.

To determine specially the quantity of acid amides in the research material (in lupin seedlings asparagin is almost the only substance present belonging to this group), about 8 gr. of dry substance are twice extracted for an hour with 40 c.c. of cold water. After filtering with the help of a suction pump, the residue is boiled once with 50 c.c. of water, and then the filtrates, and the fluid used for washing the residue, are put together.* We now rapidly boil the fluid to precipitate the dissolved proteids, filter, and boil the filtrate down to 200 c.c.

To 100 c.c. we add 10 c.c. of Hydrochloric acid, and boil for one to one and a half hours, replacing the water lost in the process; the asparagin is thus split up into Asparagic acid and ammonia. The Nitrogen of the ammonia we determine in the azotometer (see 92) by means of brominated soda solution (prepared by dissolving 100 gr. of caustic soda in 1250 c.c. of water, and treat-

* Should there be any difficulty in filtering the fluid after boiling, we pass into it for half an hour a rapid stream of washed Carbon dioxide. The filtration will then proceed very rapidly.

ing the solution when perfectly cold with 25 c.c. of Bromine). We require for the purpose 10 c.c. of the fluid. The volume of Nitrogen found is reduced to 0° C. and 760 mm. barometric pressure, and it is then easy to calculate the weight of the Nitrogen, and thence the quantity of anhydrous asparagin ($C_4 H_8 O_3 N_2$) present. Seedling extracts frequently contain small quantities of a body which at once yields Nitrogen, without previous boiling with Hydrochloric acid, on treatment with brominated soda solution. It is therefore necessary to shake up 10 c.c. of the extract in the azotometer with the soda solution directly, and to subtract the Nitrogen which may be found from that obtained after boiling the extract with H Cl.[1] The Nitrogen of the acid amides can in the above manner be determined very accurately. The calculation of this Nitrogen as asparagin is only in some measure permissible when the material, as is the case with lupins, contains only trifling quantities of amides other than asparagin.

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[1] See Sachsse, *Die Chemie und Physiologie d. Farbstoffe*, etc., 1877, p. 257, and Detmer, *Physiol.-chemische Untersuchungen über die Keimung*, etc., 1875, p. 74.

100. Behaviour of Asparagin in Plants.

If it is required to obtain information as to the physiological function of asparagin, very suitable material for investigation is provided by seedlings of Lupinus luteus. In the germination of lupins the hypocotyl elongates very considerably, the cotyledons are raised above ground, quickly strip off the seed-coat, and function as organs of assimilation. The epicotyl then at once elongates also, and the first foliage leaves unfold. The hypocotyl has developed a thick cortical parenchyma, which surrounds the circle of vascular bundles and the pith. In the stalks of the cotyledons the vascular bundles are arranged in the form of a half-moon. The ground tissue of the cotyledons is only rich in chlorophyll grains in the peripheral region. According to the microchemical researches of Pfeffer, of which I have repeated a large number, the distribution of asparagin in the seedling of Lupinus developing under normal conditions, in sunlight, is as follows. The seeds contain no asparagin. When the root has attained a length of 12 mm., and the hypocotyl a length of

2–4 mm., there is present in these organs, and in the lower plants of the stalks of the cotyledons, but little asparagin. Seedlings with roots 30 or 40 mm. in length, and whose cotyledons have not yet been thrust far above ground, contain asparagin in the root; it is, however, absent at the tip of the root. In the cortical cells of the hypocotyl, and in the lower parts of the stalks of the cotyledons, asparagin is present. It is, however, still absent in the blades of the cotyledons. When germination has advanced so far that the cotyledons are expanded, asparagin is present in them. In their stalks, and especially in the hypocotyl, very large quantities of asparagin are now present. It occurs, however, only in the cortical cells; in the elements of the vascular bundles it is, as is always the case, entirely wanting. When the epicotyl lengthens, asparagin is to be detected here also, while the other organs of the seedlings, especially the hypocotyl, gradually become poorer in asparagin. As the development of the plant proceeds, under normal conditions of vegetation, the asparagin entirely disappears from all the organs, since now, in consequence of the activity of assimilation, such large quantities of non-nitrogenous organic substances are produced, that the nitrogenous bodies formed by the dissociation of the physiological elements can at once be reworked up entirely into proteids. The fact should also be noted that, in proportion as the formation of asparagin advances during germination, the quantity of proteid reserve in the receptacles of reserve material diminishes. If, for example, we examine cotyledons of Lupinus when the elongation of the epicotyl is beginning, we find that the cell contents are already much cleared, and treatment of the sections with Iodine shows that the quantity of proteid in the cells is no longer excessive.[1]

To prove positively that the reformation of proteid from asparagin can only be effected with the help of non-nitrogenous bodies, we fill two flower-pots with garden soil or with sand, water well with food solution, and lay in them a few seeds of Lupinus luteus. The plants in one pot are grown under quite normal conditions in front of a window. The other pot is also exposed to light, but it is placed in the apparatus described in 16, and the plants grow in air deprived of Carbon dioxide. They are consequently unable to assimilate, and hence their growth is arrested when the second leaflet has unfolded. Now, and even until they die, large quantities of asparagin are to be detected in the organs

of the seedlings, especially in the hypocotyl, since the carbohydrates necessary for the regeneration of proteids could not be produced. At the time when the plants deprived of Carbon dioxide contain an abundance of asparagin, those growing under normal conditions, and constantly increasing in vigour, no longer contain asparagin, or at most only small quantities of it.[2]

If seedlings of Lupinus are grown in the dark, they die after some time, and are here again rich in asparagin, since under these conditions the non-nitrogenous material necessary for the regeneration of proteids is wanting. All this can be made out by microchemical investigation (see 98) or by analysis (see 99).

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[1] See Pfeffer, Pringsheim's *Jahrbücher*, Bd. 8.

[2] See Pfeffer, *Botan. Zeitung*, 1874, p. 249.

II. RESPIRATION.

101. General Experiments on Respiration.

THE methods to be employed in accurate researches on respiration will be discussed in the sections following. We are here at first only concerned with demonstration experiments, which will afford us a general view of the different forms of plant respiration.

We procure two wide-mouthed glass cylinders. In one we put good quantities of flowers or seedlings (wheat, peas, beans), and then close both of them with glass stoppers or corks. After a few hours we introduce into each cylinder a burning taper supported by a wire. The flame is extinguished in the cylinder provided with plant material; in the other it goes on burning. The respiring plants have used up the Oxygen of the air within the cylinder, and produced Carbon dioxide, which is unable to support combustion.

This simple lecture experiment we associate with another, which directly proves the production of Carbon dioxide as a result of normal respiration. We put together the apparatus represented in Fig. 93. The bottle, of about 10 litres capacity, and filled with water, serves as an aspirator. It is fitted with a two-holed cork, through one hole of which passes the tube *G*. This is connected up with the tube *R*, which is provided with a glass stop-cock. Before the experiment begins, the tubes *G* and *R* are filled with water. The tube *G* is in connection with *a*, *b*, *c*, and *d*. The

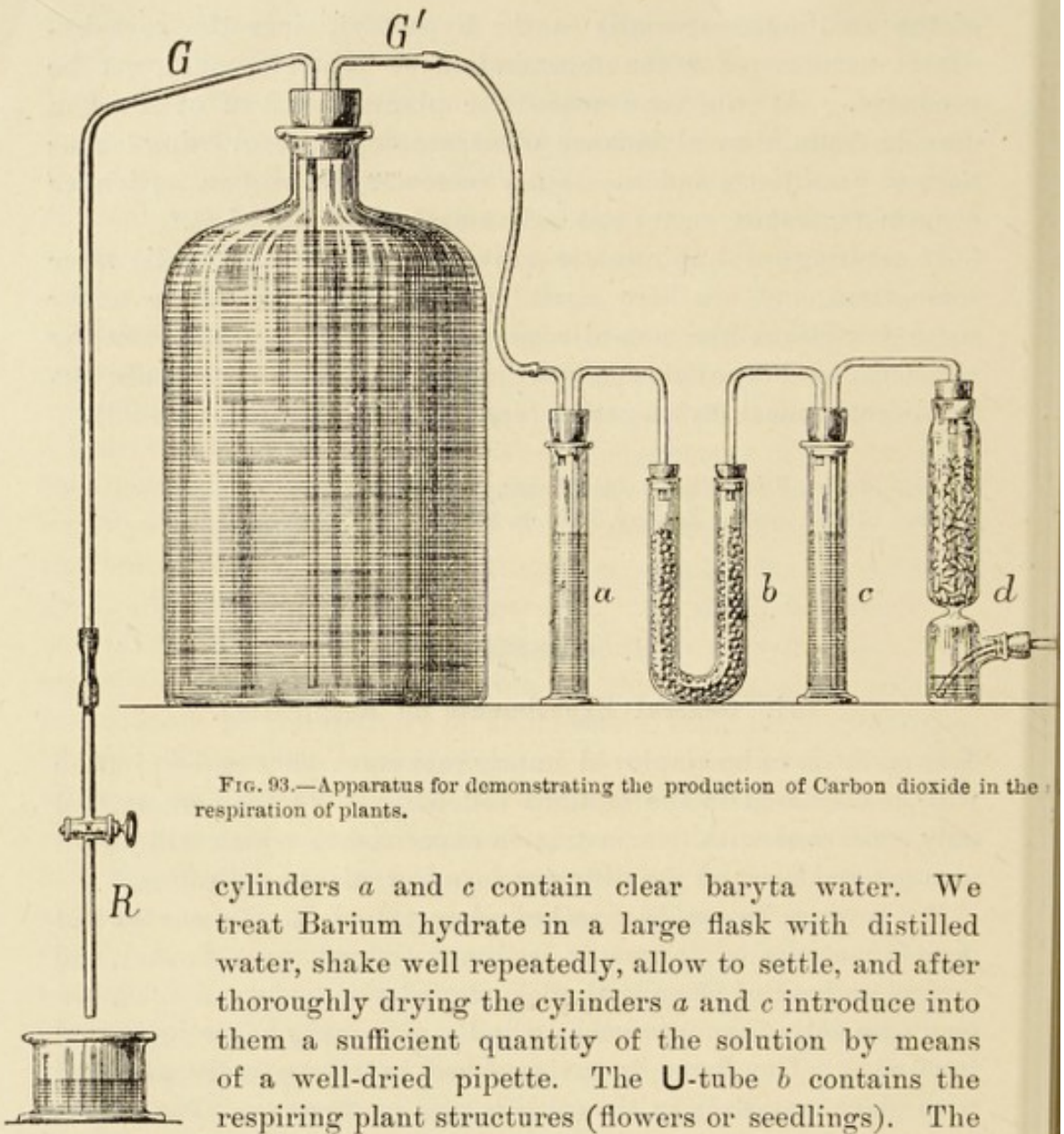

FIG. 93.—Apparatus for demonstrating the production of Carbon dioxide in the respiration of plants.

cylinders *a* and *c* contain clear baryta water. We treat Barium hydrate in a large flask with distilled water, shake well repeatedly, allow to settle, and after thoroughly drying the cylinders *a* and *c* introduce into them a sufficient quantity of the solution by means of a well-dried pipette. The U-tube *b* contains the respiring plant structures (flowers or seedlings). The lower portion of the vessel *d* contains potash solution, the upper portion fragments of caustic potash. If immediately after putting together the apparatus, we open slightly the stop-cock of the tube *R*, water flows from the tube, and a stream of air passes through the whole apparatus. It is deprived of Carbon dioxide in *d*, and hence the baryta water in *c* remains clear. The baryta water in *a*, on the other hand, very rapidly becomes turbid, or a precipitate of Barium carbonate may be thrown down, which proves that Carbon dioxide is produced by the plant material under investigation.

The following arrangement serves to demonstrate the consumption of Oxygen in normal respiration (see Fig. 94). In a wooden

support, *H*, hang two tubes which dip at the bottom into water contained in the beakers *G*, *G'*. The narrower parts *A*, *B* of the tubes, about 45 cm. in length and 15 mm. in diameter, are accurately calibrated to 0·2 c.c. The upper portions *W*, *W'*, 30 cm. long and 40 mm. in diameter, are closed with well-fitting, carefully selected rubber stoppers, through each of which passes a glass tube provided with a well-greased glass stop-cock, *h*, *h'*.* The short, wide glass test-tube seen in one of the tubes is suspended by a wire, and contains clear concentrated potash solution. Into each tube are now introduced, say, twenty-five pea seedlings, three days old, and grown at 15° C. They are placed in the upper widened part of the tubes, on moist glass wool. We put the apparatus in a place where the temperature is very constant, suck up some water into the lower portions of the tubes *A* and *B*, close the stop-cocks, and at the end of, say, half an hour, read off the position of the water in *A* and *B*. We observe also the temperature indicated by the thermometer *T*. In an experiment made by me with twenty-five pea seedlings at 15° C., the water in the tube provided with potash solution rose in twenty-one hours from 22·2 c.c. to 60·4 c.c. In the tube, on the other hand, without potash solution, if the temperature is kept approximately constant, the level of the water changes very little, because the Carbon dioxide produced in respiration appears bulk for bulk in place of the Oxygen used up. In presence of potash the water must rise, because the Carbon dioxide produced by the plants is rapidly absorbed, and does not replace the Oxygen consumed.

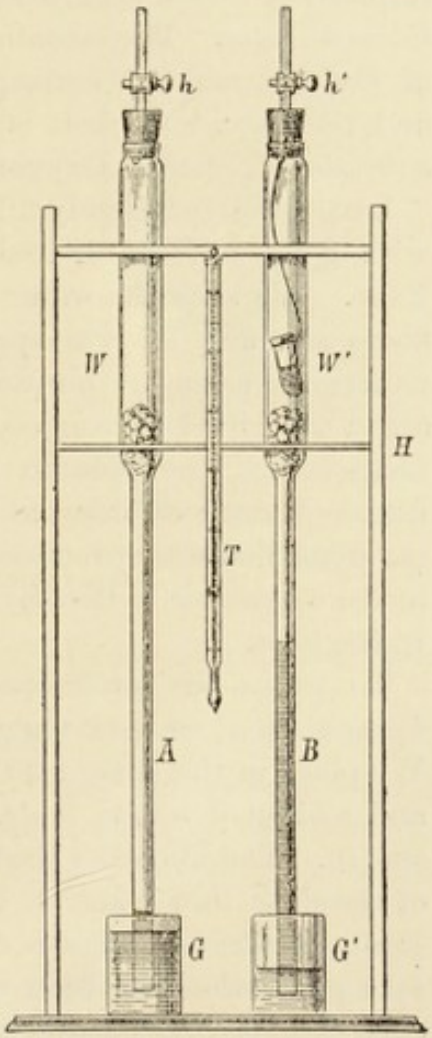

FIG. 94.—Apparatus for experiments on plant respiration.

In the following demonstration experiment also water may be used

* Instead of the rubber stoppers we may employ ground glass stoppers.

instead of mercury for the sealing fluid. Both tubes are supplied with plant material, neither of them being provided with potash solution. We employ 5 gr. of pea seeds or 5 gr. of wheat grains, and 5 gr. of hemp grains. The two lots of material are separately weighed, and after being soaked, the starchy material is placed in one of the tubes of the apparatus, the fatty material in the other. The apparatus being kept in a place in which the variations of temperature are not considerable, it will be found that the level of the water does not change much in the course of one to two days in the tube containing the starchy seeds. In the other tube, however, the water rises considerably. The fatty grains of course, like the starchy ones, undergo normal respiration; at the same time, however, the former exhibit that form of respiration which I have termed vinculatory respiration (*Vinculationsathmung*). The essential feature of this consists in absorption of Oxygen without corresponding formation of Carbon dioxide, and the bound Oxygen is employed in converting the fat into substances richer in Oxygen (carbohydrates).

Lastly, we completely fill the upper part of one of our tubes with flowers (Rosa or Dahlia, etc.), or with seedlings of Vicia Faba. We suck the water up to a considerable height, close the stop-cock, and let the apparatus stand. The material at first undergoes normal respiration. Soon, however, the Oxygen of the air in the tube is consumed, and now, even with somewhat falling temperature, the column of water begins to sink. Owing to intramolecular or internal respiration in the Oxygen-free space the plant material produces Carbon dioxide. The volume of gas in the apparatus is thereby increased, and the water is depressed in the tube.

To prove the production of Carbon dioxide (respiration) in fermentation, we put together the apparatus depicted in Fig. 95. We place in the flask *A* 200 c.c. of Pasteur's food solution (see 18), and add to it about 5 gr. of pressed yeast. Fermentation soon sets in. The liberated Carbon dioxide can be detected by means of the clear lime water or baryta water contained in the flask *B*. Fermentation and copious development of Carbon dioxide do not take place when the fluid to which the yeast is added differs in composition from Pasteur's food solution in containing no sugar, and in having Ammonium nitrate in place of the Ammonium tartrate.

We can also readily prove the formation of Carbon dioxide in

fermentation by means of Kühne's fermentation vessels * (see Fig. 96). The tube of the apparatus is completely filled with Pasteur's food solution, the bulb being left empty. We now pass a pellet of yeast into the fluid. In consequence of the evolution of Carbon dioxide which at once commences, the food solution is driven out of the tube into the bulb. If we then introduce a fragment of

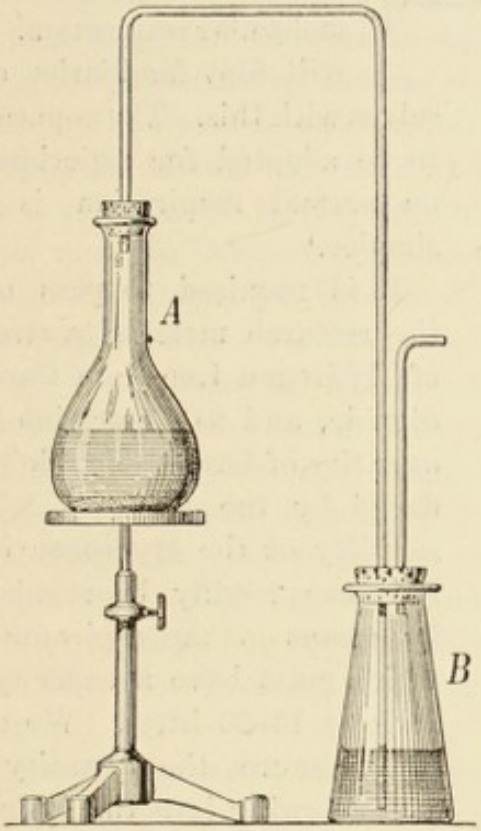

FIG. 95.—Apparatus for demonstrating the production of Carbon dioxide in fermentation.

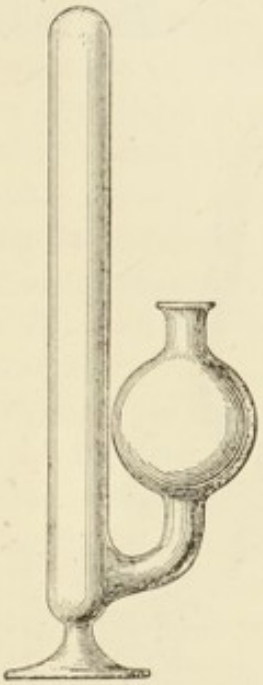

FIG. 96.—Kühne's fermentation vessel.

caustic potash into the apparatus, the fluid returns into the tube since the Carbon dioxide is absorbed.

If a tall glass cylinder is half filled with Pasteur's food solution, and, after addition of pressed yeast, is closed not quite air-tight, we can soon detect the Carbon dioxide formed by simply introducing into the cylinder a burning taper. The flame is extinguished.[1]

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[1] I have stated my views regarding normal and intramolecular respiration in my *Lehrbuch d. Pflanzenphysiologie*, Breslau, 1883. See also Detmer, Pringsheim's *Jahrbücher f. wissenschl. Botanik*, Bk. 12, and *Berichte d. Deutschen botan. Gesellschaft*, Bd. 10, p. 433.

* Any glass-blower will make them.

102. Methods for Determining the Quantity of Carbon Dioxide Produced in Intramolecular and Normal Respiration.

In Fig. 97 is depicted an arrangement which may conveniently be employed for investigating the production of Carbon dioxide in intramolecular respiration. We will first familiarise ourselves with this. The apparatus to be adopted for experiments on normal respiration is far simpler.[1]

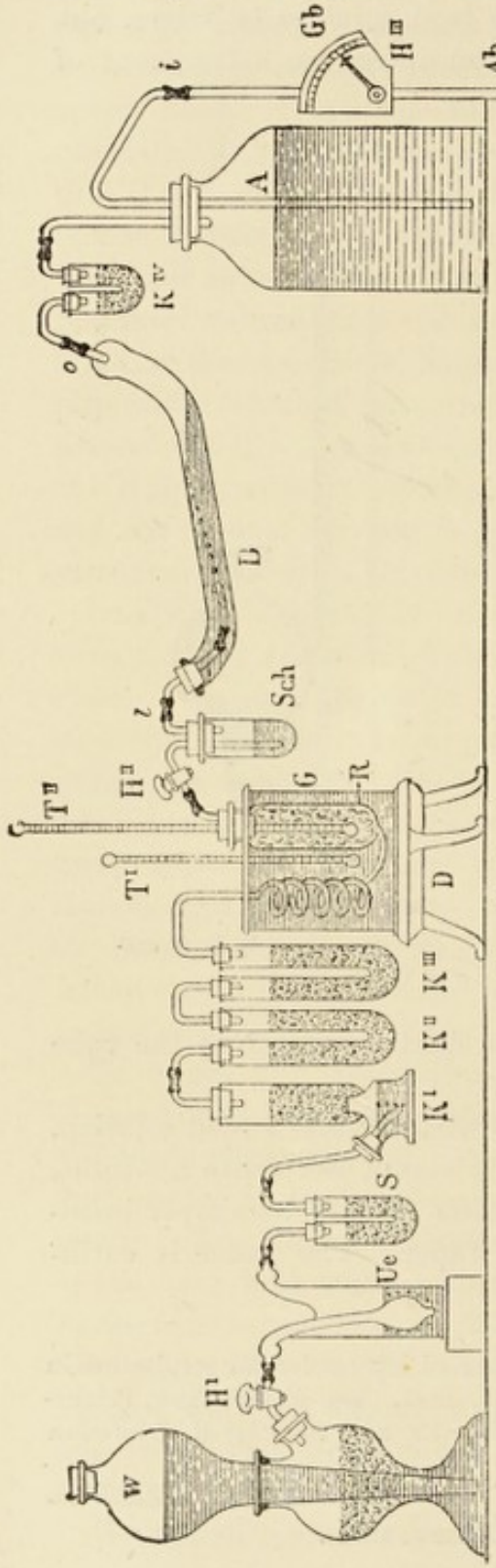

Fig. 97.—Respiratory apparatus.

It is required to pass over the research material a stream of Hydrogen free from Carbon dioxide, and to determine the quantity of Carbon dioxide produced by the material. The rapidity of the Hydrogen current can readily be regulated by means of the aspirator *A*, which must have a capacity of at least 15–20 litres. We can, *i.e.*, measure the quantity of water flowing into the cylinder *M*, *e.g.* every ten minutes, and so adjust the stop-cock H^{III} that, say, 3 litres of water run off per hour. For the sake of accurate adjustment this stop-cock is provided with a long pointer, which works over the graduated arc *Gb*. When the level of the water in the aspirator gets low, the rate of flow is reduced, and the stop-cock H^{III} has to be frequently regulated. It is hence of advantage to have the aspirator as full of water as possible,

and it can be very conveniently and rapidly filled before each experiment by connecting the off-flow tube (at the point *i*) by means of rubber tubing with the water supply. It is also advisable to use as aspirators very large vessels, *e.g.* large acid carboys.

The Hydrogen is prepared in the large Kipp's apparatus *W* from chemically pure, Arsenic-free zinc, and Hydrochloric acid free from Arsenic. In order to make sure of removing every trace of injurious admixtures, the gas is passed through the wash-bottle *Ue*, which contains a solution of Potassium permanganate, and through the U-tube *S*, containing fragments of pumice soaked with a solution of Silver nitrate. The contents of both these vessels must be frequently renewed. The Hydrogen streaming through the absorption column K^{I}, containing concentrated solution of potash below and fragments of caustic potash above, and through the U-tubes K^{II} and K^{III}, containing fragments of pumice soaked with potash solution, is deprived of every trace of Carbon dioxide. Further, we may insert between K^{III} and the worm of the respiratory vessel *R*, a small bottle containing a little concentrated Sulphuric acid, to serve as a stop valve. This valve, made like the valve *Sch*, but not represented in the figure, renders absolutely impossible any backflow of Carbon dioxide from the spiral, which however, even without this precaution, would hardly take place.

The vessel intended for the reception of the research material may have, according to requirements, a capacity of from 200 to 400 c.c., and is continued below into a spiral tube which ascends alongside the respiratory chamber proper, *R*. This last is closed at the top with a rubber stopper, through which pass a thermometer, T^{II}, and an obliquely bent glass tube. The thermometer, graduated to tenths of a degree, should have a long cylindrical bulb, which is placed in the midst of the research material. The zero of the thermometer scale must appear above the rubber stopper, so as to render it possible to read clearly when working with low temperatures. At the bottom of the respiratory chamber we place some wet glass wool, and on this the plant material, perhaps moistened.

The respiratory vessel is placed in an earthenware vessel, *G*, filled with water, provided with a wooden cover, and supported by the tripod *D*. By adding cold or warm water, or by heating with a gas flame, we can regulate the temperature of the water, as indi-

cated by the thermometer T', and hence also that of the research material in R.

The purified Hydrogen, free from Carbon dioxide, during its passage through the worm is brought to the temperature of the water. It then traverses the respiratory chamber proper from below upwards, enters the stop-valve *Sch*, which contains a little concentrated Sulphuric acid, and in the Pettenkofer's baryta tube B, containing 75 c.c. of baryta water, gives up the Carbon dioxide received from the research material. The object of the valve is to render impossible the passage of air into the respiratory space; the latter can, moreover, be completely isolated from the valve by means of the stop-cock H''. The tube K^{IV}, which contains fragments of caustic potash, is intended to prevent the passage of air containing Carbon dioxide from the aspirator into the baryta tube.

Suppose an experiment on intramolecular respiration is to be made, the apparatus is opened at l, and we pass a rapid stream of Hydrogen through it for an hour. Then l and o are connected by means of a piece of glass tubing, and the current of Hydrogen is continued for half an hour or an hour longer with the aspirator in action, during which time the temperature in R, and the rate of the current (3 l. per hour) must be carefully regulated. The Oxygen being now displaced, as thorough investigation of the method has taught, we insert the baryta tube between l and o; new tubes are put in every hour.*

For success in the experiments it is naturally of the utmost importance to have the apparatus absolutely air-tight at all points. We use only carefully selected rubber stoppers and connection tubing (the latter well greased), and well-ground glass stop-cocks, and take care that the ends of the glass tubes are in contact at the connections. The apparatus being in action, we can readily make sure that all is tight by turning the stop-cocks H' or H'''. In the former case, the flow of water at *Ab* must at once stop; in the latter the evolution of Hydrogen must cease.

To prepare the baryta water, we treat Barium hydrate and Barium chloride with distilled water (to every litre of water, 21 gr. of Barium hydrate and 3 gr. of Barium chloride). The

* To ascertain whether all the Oxygen has been driven out of the apparatus by the Hydrogen, it is only necessary to connect the valve *Sch* with a bottle containing some Phosphorus. To make sure that 75 c.c. of baryta water will completely absorb the Carbon dioxide produced, it is sufficient to join on to the baryta tube another vessel containing baryta water. This baryta keeps clear.

mixture is frequently shaken, and after some time poured into the raised bottle (Fig. 98), which has a capacity of about 10 litres. The tube k'', containing fragments of potash, serves to prevent the clear baryta water from absorbing Carbon dioxide. The solution can be run off into the burette b, which carries at its upper end the potash tube k', and the Pettenkofer's baryta tubes, after being well cleansed and thoroughly dried, are filled from the burette. The contents of these tubes are rapidly transferred at the end of each experiment to tall, well-closed cylinders, and after the precipitate has settled, we make two titrations of the clear supernatant fluid, removing 25 c.c. with a pipette for each determination. We use for the titrations (as also, of course, in titrating the original baryta water) a solution of Oxalic acid, containing 2·8636 gr. of the crystallised acid per litre, and of which 1 c.c. corresponds with 1 mgr. of Carbon dioxide. The Oxalic acid solution is admitted to the baryta water from a burette provided with a float. For the indicator, a few drops of phenolphthalein solution (100 c.c. of alcohol and 0·5 gr. of phenolphthalein) are always employed. The method enables us to determine the Carbon dioxide accurately to $\frac{1}{10}$ mgr. The above method of investigation has frequently been tested in various directions by myself and my students, and it has yielded very satisfactory results.[2] It remains, however, to be noted that the results of the observations made in the manner described are always somewhat too high. If, viz., we experiment without any

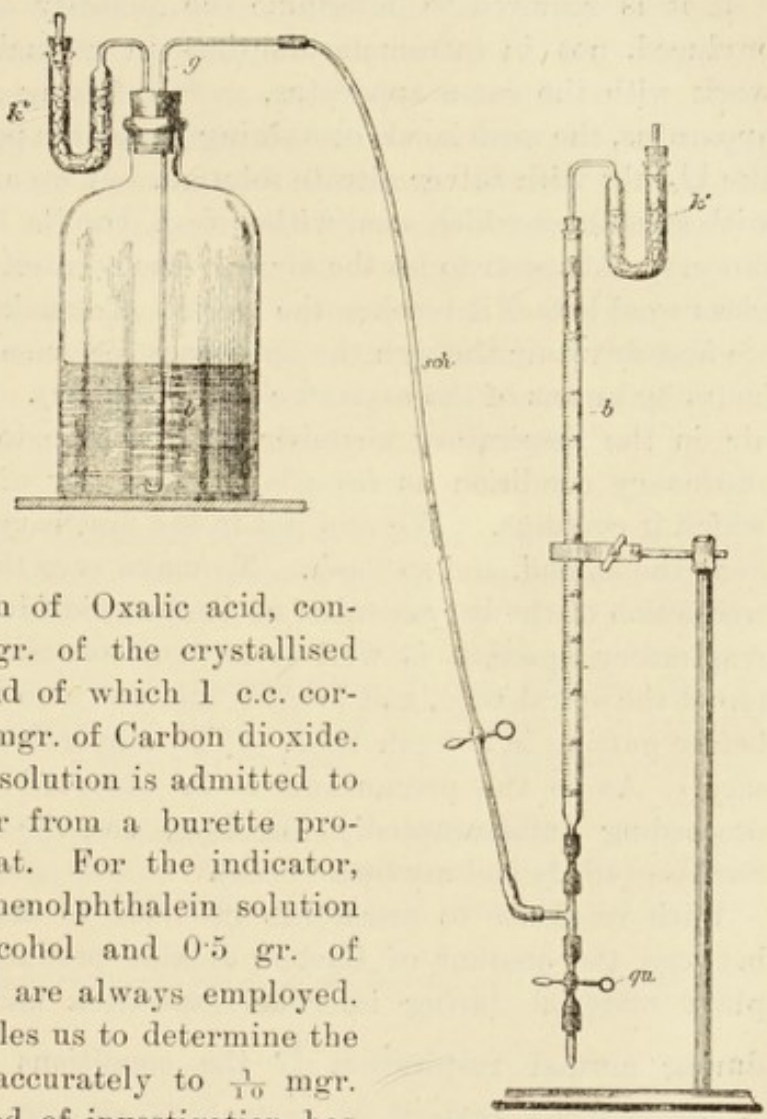

FIG. 98.—Titrating apparatus.

plant material in the apparatus, we shall still find that the titre of the baryta water has changed. This change, for 1 hour and 75 c.c. of baryta water, corresponds with 0·5 to 1·0 mgr. of Carbon dioxide. The experimental error, whose magnitude is frequently to be determined in the manner indicated, may in many cases be entirely neglected, since, as a matter of fact, the differences in Carbon dioxide evolution arising from individual peculiarities in the plants under observation are very often greater still.

If it is required to determine the quantity of Carbon dioxide produced, not in intramolecular but in normal respiration, we work with the same apparatus, merely taking away the Kipp's apparatus, the wash bottle containing Potassium permanganate, and the U-tube with Silver nitrate solution. If we are experimenting with structures which soon wither (*e.g.*, corolla leaves or delicate flowers), it is well to let the air traverse a vessel containing moist glass wool before it reaches the worm. To make the researches, we first draw air through the apparatus for one and a half to two hours by means of the aspirator (3 l. per hour). By this time the air in the respiratory chamber will be sure to have come to a stationary condition as regards the quantity of Carbon dioxide which it contains. We now put in the first baryta tube, after an hour the second, and so forth. To make sure that during the introduction of the baryta tubes no Carbon dioxide escapes from the respiratory space, it is well to have a stop-cock (or clip) at the top of the spiral tube, and to close this and also the stop-cock H'' before putting in a fresh tube. (The stop-valve *Sch* is unnecessary.) As to the precautions to be observed in investigations proceeding uninterruptedly for days, see the cited treatise of Sachsse (1872) and my own (1875).

With reference to researches intended to determine the ratio between the amount of Carbon dioxide produced by any given plant material during internal respiration and that produced during normal respiration $\frac{I}{N}$, the conditions being otherwise similar, the following particulars are to be noted:—We put together the whole apparatus, leaving out only the Hydrogen generator. Having drawn air through for one and a half hours by means of the aspirator, the determination of the amount of Carbon dioxide produced in normal respiration begins. After one to two hours, we connect up the Kipp's apparatus, pass a rapid current

of Hydrogen over the research material for an hour, then put in a baryta tube, and pass Hydrogen over the material with the help of the aspirator for an hour, at the rate of 3 litres per hour, so as to determine the amount of Carbon dioxide produced during intramolecular respiration. Finally, the Hydrogen is replaced by air, and we determine once more the intensity of the normal respiration.

In comparative researches on the respiration of plants in pure Oxygen and in air, we pass pure Oxygen over the research material from a large gasometer, instead of the Hydrogen which is necessary in investigating intramolecular respiration. The Oxygen is purified by passage through a wash bottle containing potash solution. The gas is prepared in the usual way by heating a mixture of Potassium chlorate and Manganese dioxide. It is also very convenient to fill the gasometer from a cylinder of compressed Oxygen. It is advisable to have the water intended to displace the Oxygen from the gasometer saturated with Oxygen before being so employed. Seedlings of Pisum sativum, at first at any rate, germinate as actively in pure Oxygen as in atmospheric air; other seedlings do not behave in exactly the same way.[3]

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[1] I describe here the methods which have been employed with the best results by myself and my students. See Detmer, *Physiolog. Untersuchungen über die Keimung*, Jena, 1875, and *Sitzungsber. d. Jenaischen Gesellschaft f. Medicin u. Naturwiss.*, 1881; Clausen, *Landwirthschl. Jahrb.*, 1890, Bd. 19; Amm, Pringsheim's *Jahrbücher*, Bd. 25; Detmer, *Botan. Zeitung*, 1888, and *Berichte d. Deutschen botan. Gesellschaft*, Bd. 8, 10, and 11; Ziegenbein, Pringsheim's *Jahrb.*, Bd. 25; Aereboe, Wollny's *Forschungen auf dem Gebiete der Agricultur-physik*, Bd. 16.

[2] See also Sachsse, *Ueber einige chemische Vorgänge bei der Keimung von Pisum sativum*, Leipzig, 1875; Pfeffer, *Untersuchungen aus d. botan. Institut zu Tübingen*, Bd. 1, p. 636, and Möller, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 2.

[3] See Johansen, *Untersuchungen aus d. botan. Institut zu Tübingen*, Bd. 1.

103. Carbon Dioxide Production in Normal Respiration.

After the instructions given under the previous heading, it will not be very difficult to obtain accurate results in investigating normal respiration. For practice in the method we may introduce into the respiratory vessel, say, 25 gr. of fresh petals of Rosa or some other plant, and ascertain how much Carbon dioxide they

produce per hour at a temperature of 20° C. With careful work, the results of separate experiments will present only very small differences (perhaps 1 mgr.). We may also easily prove that floral structures in general, under similar conditions, respire more vigorously than vegetative organs, *e.g.* foliage leaves.

To investigate the relationship between respiration and conditions of temperature, we determine successively the amount of Carbon dioxide produced at 0° C., 5° C., 10° C., etc. In experiments at 0° C., the respiratory vessel, together with the worm, is placed in a large vessel filled with fragments of broken ice. To obtain accurate results, the temperature must always be regulated

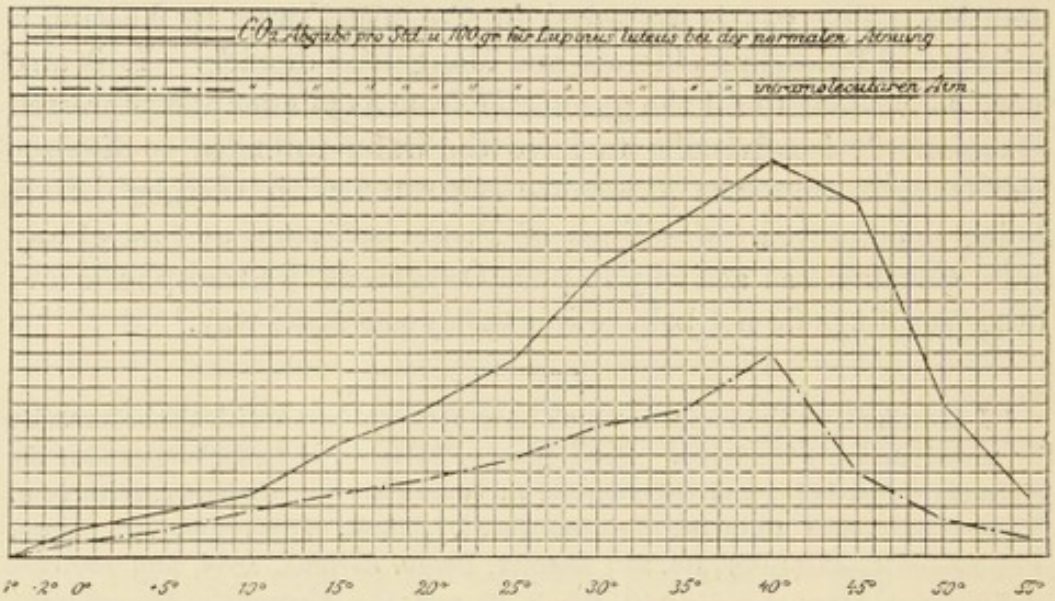

FIG. 99.—Respiration curves. (After Amm.)

during the passage of the air before introducing the baryta tube. It is very convenient to experiment with seedlings, *e.g.* seedlings of Lupinus luteus. A large number of seeds are soaked for twenty-four hours, and then germinated in sawdust, contained in suitable zinc boxes, at a temperature of 20° C. (perhaps in a warm chamber). When the seedlings are four to five days old, and have hypocotyls about 2 cm. long and roots about 3 cm. long, they are cleansed from sawdust, and may be used in quantities of 50 gr. for each experiment. Great care must be taken to have at disposal a sufficient supply of very uniformly developed seedlings. The results of the experiments are to be represented graphically in the manner indicated by the upper curve in Fig. 99. At 0° C. the seedlings of Lupinus already respire quite perceptibly. The

optimum temperature for normal respiration in Lupinus seedlings is situated at 40° C., and the maximum at 45° C. At a temperature beyond this the cells begin to die, and the production of Carbon dioxide rapidly falls.

If we first determine the intensity of respiration of 50 gr. of Lupinus seedlings at 20° C., and then expose the material in the respiratory vessel to a temperature of about 100° C. for some time, and again determine the Carbon dioxide production, we find that it has now completely ceased. Dead plants do not respire. Respiration is a function of living protoplasm. The plants must, of course, be protected from excessive loss of water while at the high temperature. It is also advisable to mix some Salicylic acid with the research material, after investigating its normal respiration, in order to prevent any subsequent development of bacteria.

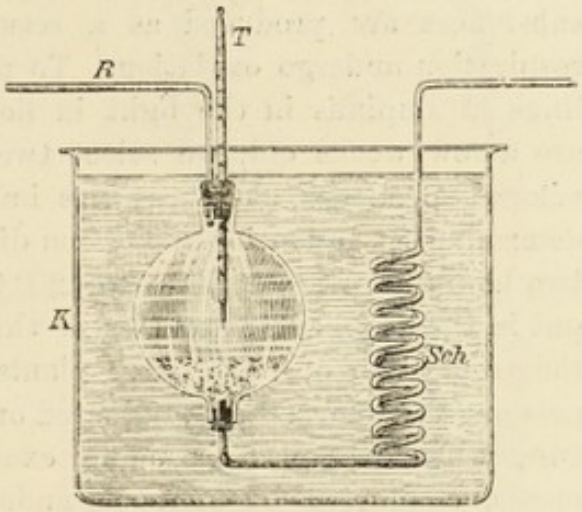

FIG. 100.—Apparatus for investigating the influence of light on respiration.

In experiments respecting the direct influence of light, we use for the respiratory chamber a vessel such as is depicted in Fig. 100. These are manufactured by Tittel & Co., Geierstal bei Wallendorf (Thuringia), and consist of a glass flask with parallel walls. The diameter of the circular faces is 13 cm., the distance between them about 20 mm. The spiral tube, *Sch*, is connected up airtight at the lower end of the vessel; the larger opening at the top receives the thermometer and the gas exit tube. The vessel is suspended in water, contained in a zinc box 30 cm. high and 10 cm. wide, the front and back of which are made of glass. This box is placed at a window with a south aspect, inclining a little backwards. In front of it, *i.e.* towards the window, is placed a large glass box, also tilted somewhat backwards, which contains a concentrated filtered solution of alum. We employ for examination perfectly chlorophyll-free floral structures, roots, or fungi (say in quantities of 25–40 gr.). In the experiments themselves the same precautions are to be observed as have already been

described above. Further information may also be obtained from the cited work of Aereboe. The utmost attention must be directed to keeping the research material constantly exposed to the same temperature during the alternate periods of light and darkness (the shading can be readily effected by covering the water-box with a suitable cardboard box, open below). Using the alum solution, this is possible, as I know from experience, even when working with direct sunlight. The experiments teach that light rays exert a photo-chemical action on respiration, probably in a very few cases only. Very generally, chlorophyll-free plants respire the same quantity of Carbon dioxide in the light as in the dark.

Indirectly, of course, the light exerts an important influence on the respiration of plants, because, through its instrumentality, substances are produced as a result of assimilation which in respiration undergo oxidation. To prove this we germinate seedlings of Lupinus in the light in flower-pots. When the plants are a few weeks old, we select twenty-five very uniformly developed specimens, cut the stems immediately above ground, and determine the quantity of Carbon dioxide which they produce in two hours at a temperature of 20° C. The flower-pots are now put in the dark, and after two or three days we again investigate the respiration of twenty-five plants. The plants in the flower-pots are then normally illuminated once more, and after four days twenty-five examples are again examined. A series of experiments, conducted by Aereboe under my direction, yielded the following results :—The twenty-five plants produced in two hours at a temperature of 20° C. :

Aug. 6, evening, 18·35 mgr. CO_2 (after illumination) ;
Aug. 9, evening, 7·95 mgr. „ (after being shaded for $2\frac{1}{2}$ days) ;
Aug. 13, evening, 18·72 mgr. „ (after being illuminated from Aug. 9 to Aug. 13).

The arrangement indicated in Fig. 101 may suitably be employed for researches on the normal respiration of roots, when it is required to determine the quantity of Carbon dioxide produced in organs vegetating normally, and still remaining in attachment with the aerial parts of the plants. A large glass cylinder placed in a water tank is used for the culture vessel, and contains a food solution. The halved cork employed to close the cylinder may be pushed in till the top of it is about 3 cm. below the rim of

the cylinder. The cork has five holes: one for the plant (*e.g.* maize); one for the thermometer, *T*; one for the dropping funnel, *Tr*, by means of which boiled-out distilled water can be added as required to the food solution; one for the in-leading tube, *Z*, which is connected with a worm, and one for the off-leading tube, *A*. To make perfectly air-tight, the space above the cork is filled with a mixture composed of wax, olive oil, and mutton suet, and melting at a comparatively low temperature. Since the culture vessel stands in the large water-box, the regulation of the temperature of the food solution in which the roots are immersed presents no special difficulty. The roots must be shaded; the aerial portions of the plant may remain exposed to the light. Before commencing an investigation, we pass a current of air through the food solution for a considerable time (say for four hours), without any preliminary determination of the amount of Carbon dioxide produced by the roots. Since the resistance of the food solution is very considerable, the stream of air must always be a fairly vigorous one (about 8–10 l. per hour). A very large bottle serves for the aspirator. The root system of the large, normally vegetating maize plant represented in Fig. 101 produced at

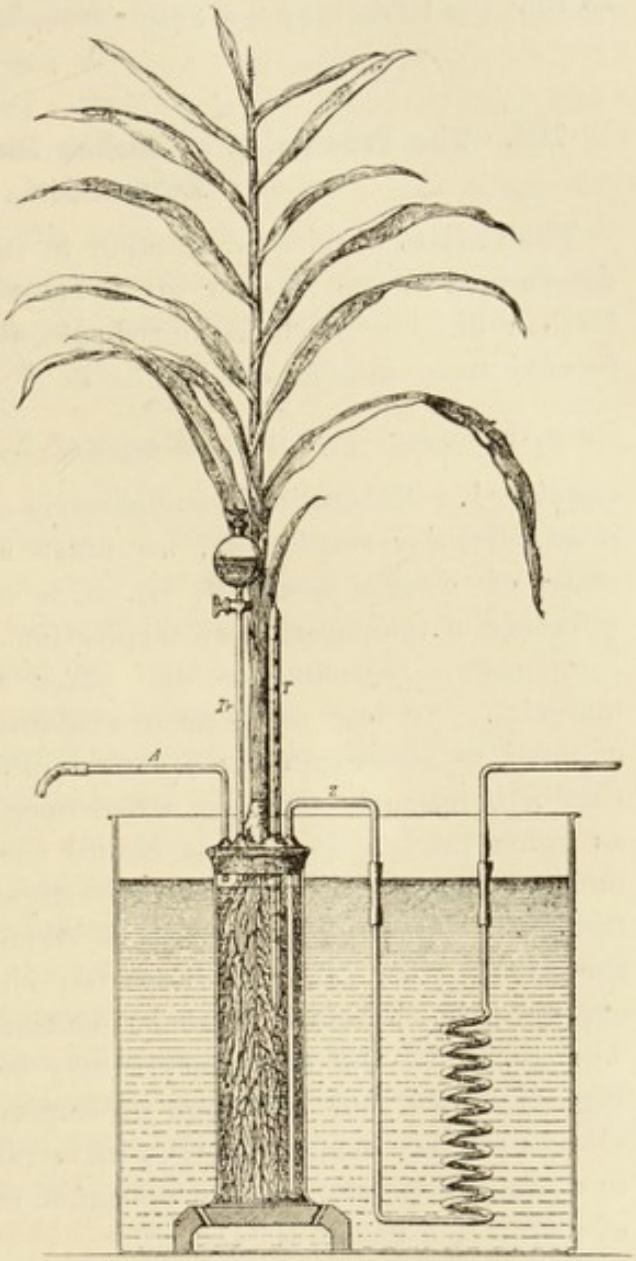

Fig. 101.—Apparatus for experiments on the respiration of roots.

20° C. 80 mgr. of CO_2 every two hours. If the plant is only to be examined off and on as to its production of Carbon dioxide, air must always be led through the food solution in the interval, so that the roots may not suffer from lack of Oxygen.

104. The Production of Carbon Dioxide in Intramolecular Respiration.

The Carbon dioxide production in intramolecular respiration is determined in the manner given below. Usually the quantity of Carbon dioxide produced intramolecularly is far less than that formed under similar conditions in normal respiration. In some cases, however (*e.g.* in seedlings of Vicia Faba), the ratio $\frac{I}{N}$ is equal to 1. Naturally, in comparative experiments on normal and intramolecular respiration, we must use material in the same state of development, if, *e.g.* it is required to determine the influence of temperature on respiration. It is best to make several comparative experiments one after the other with the same material. At low temperatures plants do not suffer injury if exposed to Hydrogen for a considerable time (many hours), and they subsequently produce, when once more respiring normally, as much Carbon dioxide as before the commencement of intramolecular respiration (see the investigations of Amm in his cited treatise). At high temperatures (from 30° or 35° C. upwards) the plants soon suffer, if kept too long in the Hydrogen; the experiments, therefore, must be continued for a few hours only. The results of the experiments may be represented graphically. Fig. 99 shows that seedlings of Lupinus (four or five days old) always produce much less CO_2 in intramolecular respiration than in normal respiration. The optimum temperature for both kinds of respiration lies at 40° C.*

Intramolecular respiration takes place even when plants are introduced into a vacuum.

* This latter result, first determined by Amm, was confirmed by Chudiakow (*Landwirthschl. Jahrbücher*, Bd. 23). He disputes, however, the accuracy of some conclusions of Amm and myself. We had found that the ratio $\frac{I}{N}$ is not the same at all temperatures, and this statement Chudiakow, on the ground of the results of his investigations, controverts. His work, however, is not, to my mind, conclusive, and further researches are necessary to settle the matter.

Experiments *in vacuo*, which I, for example, made with pea seedlings after Wortmann's method,[1] are carried out as follows:—A thick glass tube, fused up at one end, and about 100 cm. long and 1·5 cm. in diameter, is filled with clean and perfectly dry mercury. As to the method of cleansing mercury, see 13. To prevent bubbles of air from adhering to the walls of the tube in filling it, it is best to run the mercury, by means of a funnel rather finely drawn out at the end, through a thin glass tube reaching to the bottom of the tube to be filled. When the tube has been filled, it is closed, and inverted in a flat glass vessel partially filled with mercury. We now have before us a barometer with a fairly large Torricellian vacuum. A few seedlings, developed in moist sawdust, are freed from the seed-coats, dried with blotting-paper, and passed up through the mercury of the barometer tube, together with a little ball of blotting-paper soaked in boiled-out water, which serves to keep them moist. In experiments with Pisum sativum or Vicia Faba, we employ six to ten seedlings; if we experiment with lighter seedlings, a correspondingly greater number must be used. When the mercury has come to rest in the barometer tube, after introduction of the seedlings, we at once proceed to observe the time, the temperature, and the barometer reading, and also the level of the column of mercury (the upper level and the lower level, *i.e.* the point at which the barometer tube touches the mercury in the flat vessel). If we work with non-graduated barometer tubes, we mark the upper and lower limits of the column of mercury by pasting strips of paper on the tube, repeating this at each successive reading, measure the heights of the columns of mercury thus indicated, and determine later the volumes corresponding to them by running mercury from a burette up to the corresponding marks. All the volumes are reduced to 0° C. and 1,000 mm. of mercury.

If at the commencement of the experiment:

Vo = the volume,

h = the height of the Hg. in the barometer tube,

t = the temperature, and

b = the barometer reading,

and further, if

ts = the tension of the aqueous vapour over the mercury in the tube at the corresponding temperature, and

a = the coefficient of expansion of air, the reduced volume V is given by the following equation:—

$$V = \frac{b-(h+ts)}{1{,}000} \times \frac{Vo}{(1+at)}.$$

If V was the volume at the commencement of the experiment, and V_1 the volume calculated at the end of say six hours, then $V_1 - V$ is the volume of Carbon dioxide given off during that time. Since *in vacuo* the initial volume was zero, V_1 directly indicates the volume of Carbon dioxide evolved.

It is often desirable to be able to compare the rate of intramolecular respiration with that of normal respiration, and in order to do this we must arrange beside the barometer tube a second glass tube of the same size, and into this introduce seedlings as nearly as possible of the weight and in the same stage of development as those in the vacuum. To prevent them falling back, we push a small cork down the tube. This is then inverted in mercury, and about 20 c.c. of the atmospheric air present in it is removed by suction, the mercury naturally rising to that extent (see Fig. 102). To so remove a portion of the air from the tube we use a glass flask, closed by a rubber stopper, through which passes a bent glass tube carrying a piece of rubber tubing. We warm the flask, close the tubing with a clip, and then introduce the end of it into the tube. The flask is allowed to cool, and on releasing the clip it acts as a suction apparatus, by means of which the mercury is easily caused to ascend in the tube. We now finally cover the mercury in the tube with a layer of water about 3 mm. thick, make the necessary readings for determining the volume of air in the apparatus, and introduce a fragment of caustic potash into the tube. The

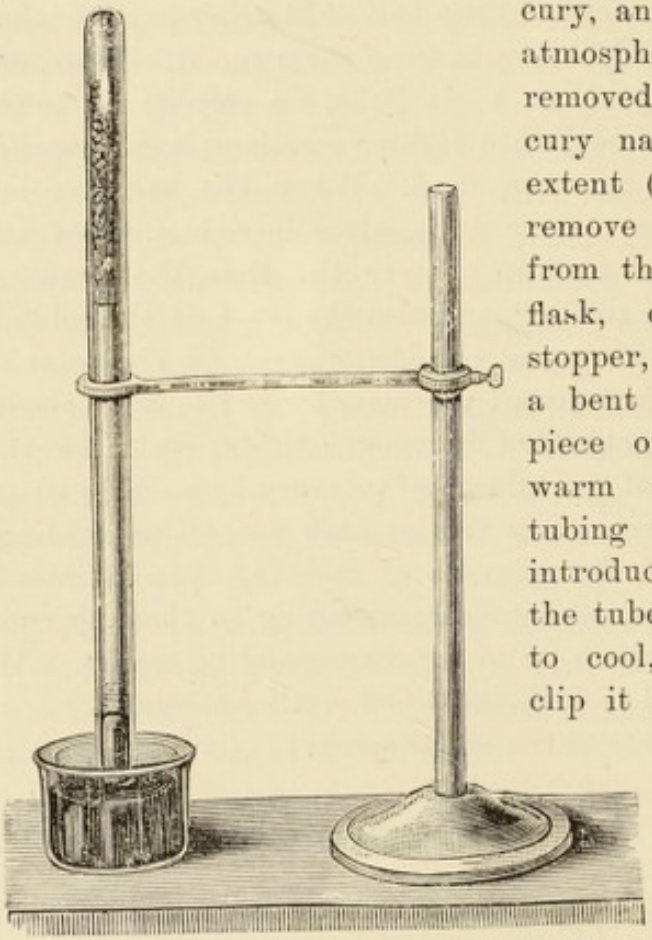

Fig. 102.—Apparatus for determining the quantity of Oxygen which plant structures can take up in respiration.

potash is rapidly dissolved by the water above the mercury, and since the potash solution formed absorbs the Carbon dioxide developed by the normal respiration of the seedlings, we can at any time make fresh readings to determine the amount of Carbon dioxide produced. This method, it is true, does not by any means afford such accurate results as that indicated in 102.

If we allow the experiments on intramolecular respiration to proceed for a considerable time, *e.g.* a few days, we shall find that a given quantity of seedlings, in unit time and under constant external conditions, produce less and less Carbon dioxide. The seedlings gradually pass into a pathological condition, and it is important to know this, because it follows that in comparative researches on intramolecular and normal respiration we must not take too long an experimental period (only about six to eight hours). Such comparative experiments teach, further, that only a few plants, *e.g.* seedlings of Vicia Faba, produce as much Carbon dioxide in intramolecular as in normal respiration. Most plants give off much larger quantities of Carbon dioxide in presence of Oxygen than when Oxygen is absent.

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[1] See Wortmann, *Arbeiten d. botan. Instituts in Würzburg*, Bd. 2.

105. Analytical Researches on Respiration.

In order to deal with many questions relating to respiration, it is essential to determine the quantity of Carbon, Hydrogen, and Oxygen in the research material at the beginning of the experiments and at their close. If, *e.g.*, we ascertain the absolute quantity of these elements contained in 100 gr. of seeds, and in the seedlings produced after a certain time from 100 gr. of seeds, we obtain comparable numbers which at once indicate the quantity of Carbon, Hydrogen, etc., consumed in respiration. Such ultimate analytical researches are of special significance if, *e.g.*, we are dealing with the question whether the whole of the Carbon lost by germinating seeds is given off in the form of Carbon dioxide, or whether in germination other carbonaceous gases are produced (Carbon monoxide, Hydrocarbons). In such researches we compare the results of direct Carbon dioxide determinations, which are to be made in the manner indicated in 102, with the results of the analyses of seeds and seedlings. If there is a close agreement between the numbers obtained from the respiratory investigations of the loss of Carbon

in germination, and those afforded by analytic investigations, we are justified in the conclusion that the whole of the Carbon leaves the germinating seeds in combination with Oxygen as Carbon dioxide. For details as to the method of procedure the treatises cited in the footnote must be consulted. Respecting the ultimate analyses, to secure success in which much practice is essential, I will only observe further that the seed and seedling substance, in consideration of the Chlorine and Sulphur contained in it, must be mixed before the combustion with Lead chromate, and that in consideration of the Nitrogen contained in it, metallic Copper (copper turnings) must be placed at the front end of the tube. The combustion itself is best performed in a stream of Oxygen.[1]

[1] See Sachsse, *Ueber einige chemische Vorgänge bei der Keimung von Pisum sativum*, Leipzig, 1872; Detmer, *Physiologische Untersuchungen über die Keimung ölhaltiger Samen*, etc., Jena, 1875.

106. Absorption of Oxygen in Respiration, and Determination of the Respiratory Ratio $\frac{CO_2}{O}$.[1]

For researches whose object is to determine the quantity of Oxygen absorbed, and at the same time the quantity of Carbon dioxide produced, in normal respiration, the apparatus represented in Fig. 103 may be employed.

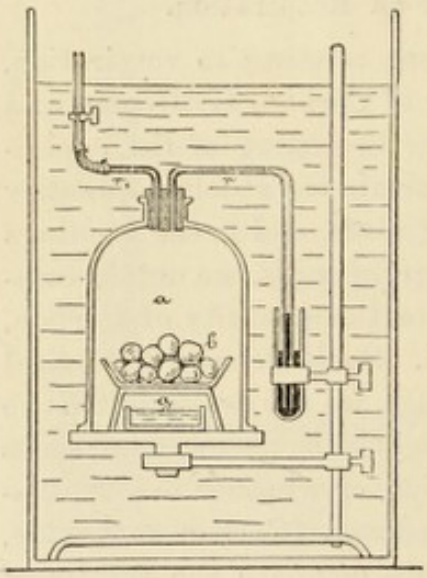

Fig. 103.—Apparatus, after Stich, for determining the amount of Oxygen absorbed and of Carbon dioxide produced in the respiration of plants.

The bell-glass, *a*, about 800 c.c. in capacity, and provided with a carefully ground broad rim, rests on a ground glass plate. The rim of the bell-glass is well greased with a mixture prepared by melting together 1 part of wax and 3 parts of lard. The tubulus of the bell-glass is fitted air-tight with a rubber stopper, which receives the tubes r_1 and r. The tube r_1 is provided with a stop-cock, and serves to put the interior of the bell-glass in communication with the atmosphere. The long limb of the tube r, which has an internal diameter of 7 mm., and is graduated to

$\frac{1}{10}$ c.c., dips into a vessel containing mercury. The porcelain dish, *b*, the bottom of which is perforated, contains the research material, resting on moist filter paper or moist glass wool. It is supported on a glass tripod. The shallow glass, *g*, contains potash solution to absorb the Carbon dioxide evolved. The whole arrangement, supported on a nickel-plated stand, is placed when in use in a glass case, the front and back of which are parallel to one another, and which is filled with water. We are thus enabled to conveniently regulate the temperature, and keep it constant.

At the commencement of an experiment we draw the mercury a little way up the tube *r* by suction at r_1, close the well-greased stop-cock, and then wait for about half an hour before proceeding to read the position of the mercury in *r*. The readings must always be made by means of a telescope. We naturally determine also the length of the column of mercury, together with the temperature and barometric pressure. It is necessary to know the capacity of the bell-glass, of the tube *r*, and of the tube r_1 as far as the stop-cock. From this volume is to be deducted, however, the aggregate volume of the objects in the apparatus (dish containing potash solution, glass tripod, porcelain dish, moist filter paper, research material). The volume of these we ascertain in part by immersion in water contained in a graduated vessel. Great stress is to be laid on accurate readings of temperature and barometric pressure. The reduction of the volumes can easily be effected by employing the formula given in 104.

The most important source of error in the method lies in the fact that at the commencement of the experiments we cannot at once make the readings, but have to wait for about half an hour till the temperature is in equilibrium. But during this half-hour the plants are already taking up Oxygen, and to make allowance for this, we correct the actually found volume of Oxygen absorbed by calculating the quantity corresponding with half an hour, and adding this to it. This correction is naturally a near approximation only when the research material during the period of observation does not undergo any important change in its rate of respiration.

The potash solution employed for absorbing the Carbon dioxide is accurately weighed. It must be nearly concentrated and perfectly clear. At the end of each experiment we pour the potash solution into a small flask, dilute with water, precipitate the Carbonic acid with $BaCl_2$, and filter. To wash the precipitate on

the filter, we first use water saturated with $BaCO_3$, then pure water. The Barium carbonate, after being dried and gently ignited, is weighed, and we calculate the corresponding volume of Carbon dioxide at 0° C. and 1,000 mm. barometric pressure. The potash solution employed always contains more or less Potassium carbonate to begin with, and the Carbon dioxide of this must be determined by special experiments, and taken into account.*

With the apparatus described we can deal with many physiological questions. Above all it is instructive to follow closely the respiration of germinating seeds. To make the experiments we soak, and lay on the moist blotting-paper, say 2 gr. of wheat grains, 4 gr. of peas, or 1 gr. of Raphanus sativus seeds. After every twenty-four hours the apparatus is opened, the potash solution replaced by new, and the observation continued. The temperature must always be kept very constant, *e.g.* at 15° or 20° C.

In experiments with wheat we find, *e.g.*, that 2–2·5 gr. of grains with advancing germination also absorb increasing quantities of Oxygen, and produce increasing quantities of Carbon dioxide. From the fifth day onwards, at a temperature of 20° C., about 20 c.c. of Carbon dioxide are produced, and 20 c.c. of Oxygen absorbed, every twenty-four hours. The respiratory ratio is here (and generally in starchy plant structures) approximately = 1. In the germination of fatty seeds (*e.g.* of Raphanus) we find the ratio = 0·6–0·8. They take up relatively much Oxygen, because the fat in germination suffers oxidation, and gives rise to carbohydrates.

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[1] Literature : Detmer, *Physiol.-chem. Unters. über Keimung*, 1875; Godlewski, Pringsheim's *Jahrb.*, Bd. 13 ; Moeller, *Berichte d. Deutschen botan. Gesellsch.*, Bd. 2; Stich, Flora, 1891 ; Bonnier and Mangin, *Annal d. sc. nat.*, Sér. 6, T. 17 and 18, give a method of gas analysis, employed also for example by Stich, which in many cases is very serviceable. Further see Bonnier and Mangin, in *Annal. d. sc. nat.*, Sér. 6, T. 19 ; Sér. 7, T. 2.

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* It is still better to determine the Carbon dioxide by the method of titration (see 102). The CO_2 must then naturally be precipitated from the potash solution before and after the experiment by means of $BaCl_2$, the fluid remaining behind being subjected to titration.

107. The Behaviour of Plants in Contact with Nitrous Oxide Gas.

It has often been asserted that plant-cells are able to utilise the Oxygen of Nitrous oxide for normal respiration. I have made this question the subject of special investigation,[1] and my experiments have been made as follows. A retort-like vessel (see Fig. 11) of about 90 c.c. capacity was filled with distilled water which had been well boiled and then allowed to cool completely in a closed vessel, and the water was then displaced by Nitrous oxide, *a*. A second retort, *b*, was similarly filled with boiled-out water, which was replaced by N_2O after the introduction of twenty seven-days-old pea seedlings raised in the dark. A third retort, *c*, was likewise supplied with pea seedlings and water, but the water was displaced by atmospheric air. The N_2O is prepared by heating commercial Ammonium nitrate in a retort, and the evolved gas is led before use through a solution of Ferrous sulphate, and through potash, to free it from the small quantities of Nitric oxide and Nitric acid which may be present. In introducing the gas, care must be taken that a very small quantity of water is left behind in the tube of the retorts. In my experiments the retorts were left for twenty hours at a temperature of about 20° C. The retort tubes dipped into mercury, and the small quantities of water left in the tubes served to protect the seedlings from the injurious effect of the mercury vapour. At the end of the twenty hours all the retorts were placed, still inverted, with their mouths under water cooled by means of broken ice, care being taken not to admit air during the transference. The gas present in the retorts *a* and *b* now gradually became almost completely absorbed, while in the retort *c* a large volume of gas remained behind. The N_2O therefore could not have been decomposed by the seedlings. The small quantities of gas remaining, after the absorption of N_2O, in the retort *a* (seedlings absent), and in the retort *b* (seedlings present), were clearly derived from the water used for absorption.*

We further make the following experiment to show that seeds are unable to germinate in Nitrous oxide gas. Two retort-like vessels, *a* and *b*, are filled with distilled water, which has been

* The preparation of the N_2O must of course be allowed to proceed for some time before the gas is introduced into the vessels, so as to drive all the air out of the generating apparatus. At the close of the experiments we may transfer the vessels to cold alcohol instead of cold water, since the former absorbs the N_2O more energetically.

boiled out and then allowed to cool thoroughly in closed vessels. In each vessel we now place a few soaked wheat grains, dip the mouths of the vessels under mercury, and replace the water in *a* by N_2O, that in *b* by atmospheric air. In the course of a few days the grains in *b* germinate; those in *a* do not germinate. If, however, they are placed in the air, and exposed to normal conditions of germination, they subsequently develop, if they have not been kept in the Nitrous oxide too long, *e.g.* for only two days.

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[1] See Detmer, *Landwirthschl. Jahrbücher*, Bd. 11, p. 213. See also Möller, *Ber. d. Deutschen botan. Gesellsch.*, Bd. 2.

108. Formation of Alcohol in Plants, and the Behaviour of Anaërobic Organisms.

We procure some wort from a brewery, transfer say 200 c.c. of it to a flask, and add a small quantity of very pure yeast which has been made into a pulp with water. The dry weight of the yeast we determine by a control experiment. After some time, when the fermentation, which quickly becomes energetic, has slackened, we collect the yeast in the flask on a weighed filter, and find by determination of its dry weight that a considerable production of yeast has taken place under the conditions described.

Saccharomyces cerevisiæ grows actively if well supplied with Oxygen. But under these conditions, as also with a limited supply of air or complete deprivation of Oxygen, the fungus is able to provoke in certain food solutions very vigorous alcoholic fermentation, as is readily proved by aspirating air through wort to which a fair quantity of yeast has been added, or even by leading Oxygen into it from a gasometer.

In this and in many other cases it is of importance to obtain a measure of the rate of fermentation. This is secured by determining the quantity of sugar decomposed in the fermentation, or the quantity of alcohol produced.

In the sugar determinations, 10 c.c. of the Pasteur's solution, for example, with which we experimented, is diluted till the proportion of sugar is reduced to $\frac{1}{4}$–$\frac{1}{2}$ per cent., and if cane-sugar has been used for making the Pasteur's solution, is heated for some time with Sulphuric acid. (See above under sugar determination.) The determinations of sugar are then easily made by means of

Fehling's solution. The fermented fluid is in every case to be heated for a considerable time in a state of dilution before the sugar determinations are made, in order to drive off the alcohol.

Quantitative determinations of alcohol are made as follows:—200 c.c. of the fermented fluid, without being filtered, is distilled in a flask of 400 c.c. capacity. To prevent the fluid from frothing up, we put into it a fragment of paraffin. The flask in which the distillate is collected must be closed almost air-tight, so as to prevent loss of alcohol. During the distillation, the receiver must be kept cool by means of a stream of cold water. When we have obtained about 100 c.c. of distillate, we determine its specific gravity. From this, by means of suitable tables (see König, *Untersuchung landwirthschaftlich und gewerblich wichtiger Stoffe*, Tabelle 15), the quantity of alcohol contained in 100 c.c. of the distillate can be directly deduced in percentage by weight or volume. The number found must be divided by 2, since the distillate contains the whole of the alcohol of the original 200 c.c. of fermented fluid.

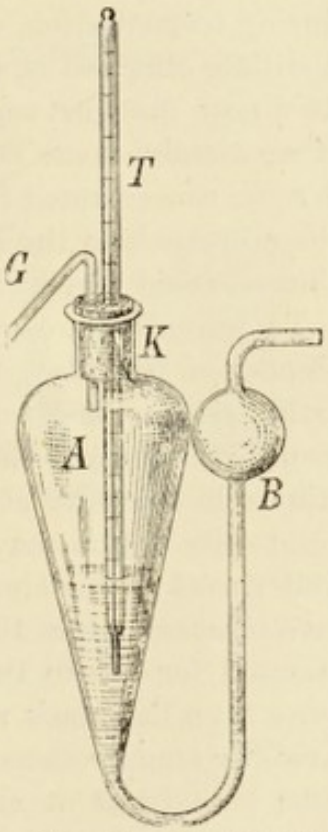

FIG. 104.—Apparatus for investigating fermentation.

If it is required to lead gases (air, Hydrogen) through the fermenting food solutions, we may use the apparatus represented in Fig. 104. The funnel-shaped portion, *A*, which receives the food solution, is continuous with the glass tube, *B*, on which is blown a bulb. The rubber stopper, *K*, receives the thermometer, *T*, and the gas exit tube, *G*. Through *B* gas is introduced into the fluid, through which it streams from below upwards. The portion *A* of the apparatus has a capacity of about 250 c.c. In order not to lose the alcohol carried over with the gas, *G* is connected with a condensing apparatus. This consists of a small flask, which can be closed by means of a two-holed cork. Into one hole passes a tube to carry off the gas, while the other receives the tube bringing the gas, the portion of this within the flask being in the form of a worm. The lower end of the worm dips into some water contained in the flask. The condensing apparatus is placed in a large glass con-

taining water and broken ice. If an experiment is to be interrupted before the fermentation is completed, in order, *e.g.*, to determine the quantity of alcohol produced at the end of a definite time, we open the fermentation vessel and add to the food solution 20 c.c. of 5 per cent. corrosive sublimate solution, so as to secure instantaneous cessation of the fermentation.

The apparatus here described is very serviceable, *e.g.*, when it is required to prove that yeast brings about vigorous fermentation in wort, both in absence of Oxygen and in presence of air, while with Pasteur's food solution the fermentation is only feeble in presence of air, but active in absence of Oxygen. Regarding the yeast to be employed, consult the appendix. To the food solution in *A* must be added a few c.c. of yeast fluid.[1]

If it is only desired to prove qualitatively that alcohol is formed during fermentation, we proceed in exactly the same way. The distillate obtained is, however, again subjected to distillation. The fluid now obtained smells strongly of alcohol; it is inflammable. If we dissolve some Potassium bichromate in a little water, add to it some concentrated Sulphuric acid, and introduce a few drops of the mixture into the last distillate, it becomes green, because the Chromic acid is reduced, the alcohol present being oxidised.

To prove the very important fact that the higher plants in absence of free Oxygen, while they undergo intramolecular respiration and at last gradually perish, produce alcohol, we experiment with grapes, cherries, or peas. The objects are laid for a minute in corrosive sublimate solution (1:1,000), so as to kill any yeast-cells which may be clinging to them externally, and then well rinsed with water. The fruits are now at once used for the investigation, while the pea seeds are soaked in water, and germinated for one to two days on moist blotting-paper.* We now quite fill a litre flask with the fruits or seedlings and close it with a rubber stopper through which passes the shorter limb of a glass tube bent twice at right angles. The longer limb of this tube dips into mercury. The Oxygen in the apparatus is soon consumed. Intramolecular respiration speedily sets in, which results in an evolution of gas continuing often for weeks, but ultimately becoming slow, and at last completely ceasing, when the objects are dead. If in this state brought into the air, they rapidly undergo decomposition. When the fruits or seedlings have been

* The water and blotting-paper must first of all be sterilised.

in the apparatus for three to four weeks, we open it, pound the material, and subject it (in the case of the seedlings after addition of water) to distillation. The fruits yield perhaps $1\frac{1}{2}$ per cent. (in terms of the weight of fresh material taken), the pea seedlings 5 per cent. of their dry weight of alcohol. The alcohol can easily be recognised as such in the manner above indicated. Aromatic compounds and fusel oil are always mixed in larger or smaller quantities with the alcohol produced.*

Yeast, as shown particularly by Pasteur's valuable researches, is able to grow not only in presence of air, but also, though more slowly, in complete absence of Oxygen. On the other hand, the Butyric acid organism (Clostridium butyricum, a Schizomycete) is one of the obligate anaërobia. The fungus appears in the form

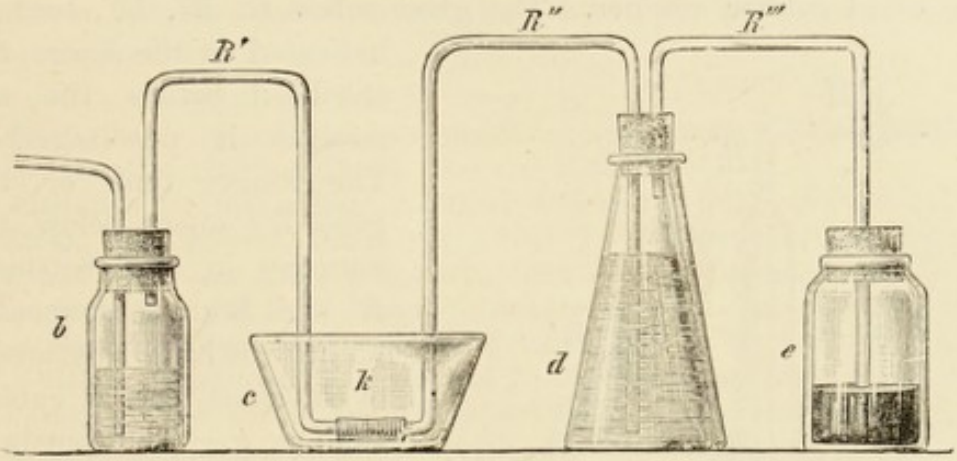

FIG. 105.—Apparatus for proving that there are organisms capable of growing in complete absence of Oxygen.

of shorter and longer rodlets. We prepare a 5 per cent. solution of cane-sugar, to which we add some meat extract, so that the fluid appears yellowish. We also add to the fluid some Potassium carbonate, so as to give it a slightly alkaline reaction. Still more suitable is the following food solution:—to one litre of water 50 gr. of potato starch, 0·5 gr. of Ammonium chloride, 0·2 gr. of Magnesium sulphate, 1 gr. of acid Potassium phosphate, and 25 gr. of chalk. If the solutions are left in the thermostat at 35° C., in not too tightly corked thick-walled vessels, they exhibit by the

* The formation of alcohol, according to our present views, appears to be actually a function of the living protoplasm. The whole subject, however, needs further very searching experimental treatment. We must particularly keep in view the injuries which plant-cells experience when kept for a considerable time in a space devoid of Oxygen.

second or third day active fermentation. The evolution of gas (CO_2 and H) resulting from the spontaneous appearance of the butyric organism is so energetic, as I have observed, that the corks are forcibly expelled, and the fluids at once acquire the characteristic smell of Butyric acid. We will now demonstrate the anaërobic character of Clostridium butyricum, and for that purpose put together the apparatus represented in Fig. 105.

The flask *d* of about 500 c.c. capacity is about two-thirds filled with food solution of the composition indicated. We plug the mouth of the flask with cotton wool, and sterilise the solution by boiling for about half an hour. After cooling, we remove the cotton wool, quickly infect the contents of the flask with a few drops of a fluid in which the butyric fermentation is already proceeding, and at once close the flask with a very well-fitting, two-holed rubber stopper. The glass tubes *R'*, *R''*, *R'''*, bent as indicated in the figure, are sterilised before the apparatus is put together. The longer limb of the tube *R'''* opens below the mercury in the bottle *e*. *R'* and *R''* are connected within the basin *c* by means of the short piece of rubber tubing, *k*. The bottle *b* contains a solution of Potassium permanganate. We now prepare Hydrogen by the action of Arsenic-free Zinc on Hydrochloric acid (the water used to dilute this must first be boiled, so as to free it as completely as possible from air), and let it enter the apparatus at *b*, after passing through a wash bottle containing potash solution. This wash bottle and the Kipp's apparatus are not represented in the figure (but see Fig. 97). We lead Hydrogen through the apparatus for about two hours, so as to displace all the Oxygen, then pour mercury into the dish *c* and remove the connection tubing *k*, so that the tube *R''* now opens under mercury. The apparatus is put in a thermostat at 35° C. In the course of a few

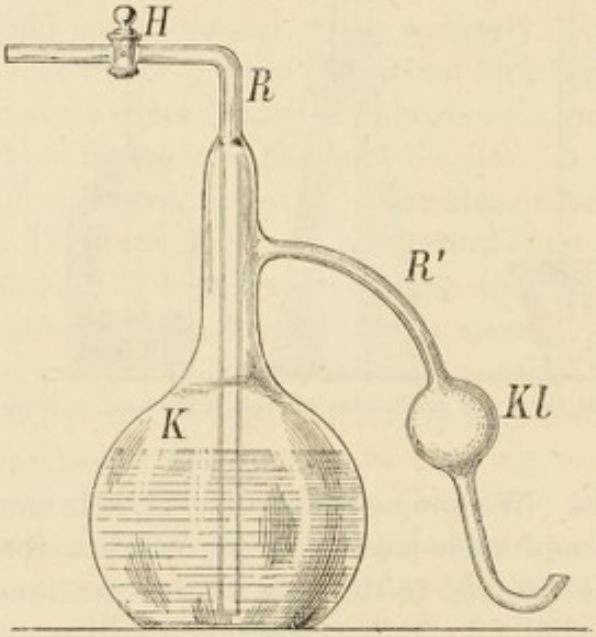

Fig. 106.—Apparatus for investigating fermentation in absence of free Oxygen.

days fermentation begins. The fluid in *d* becomes more and more turbid, and we thus see that the Butyric acid organism is able to develop in complete absence of free atmospheric Oxygen.[2] To make the experiment with yeast we use wort as the food solution, and infect it with the smallest possible quantity of pure yeast.

It is also very convenient to work with the apparatus represented in Fig. 106. The flask *K* holds about 250 c.c. We fill it with food solution and sterilise this by boiling. After cooling the solution we introduce into it, by passing a fine glass tube through the leading tube *R*, which is provided with a stop-cock, a small quantity of fluid containing Clostridium or yeast-cells, pass a current of Hydrogen for two hours, put the end of the tube *R'* under mercury, and at once close the stop-cock *H*. The bulb *Kl* has merely the object of rendering it impossible for mercury to pass over into the flask if the temperature of the laboratory should fall considerably.

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[1] For further information about fermentation experiments, see Chudiakow in *Landwirthschl. Jahrbücher*, Bd. 23. See also Note 2.

[2] Literature for the section: A. Mayer, *Lehrbuch d. Gährungschemie*, 1874, Nachtrag, 1876; Pasteur, *Compt. rend.*, 1861, T. 52; also in the years 1863, 1872, and 1875; also *Etude s. l. bière*, 1876; Brefeld, *Landwirthschl. Jahrbücher*, 1874, 1875, and 1876. On the formation of alcohol in the cells of the higher plants, see Brefeld, *Landwirthschl. Jahrb.*, 1876, p. 324, and Lechartier and Bellamy, *Compt. rend.*, T. 69, 75, and 79. Respecting anaërobic organisms, see Pasteur's cited papers, and Prazmowski, *Unters. über einige Bacterienarten*, Leipzig, 1880; Detmer, *Lehrbuch der Pflanzenphysiologie*, 1883, p. 173.

109. Production of Heat in Plants. Phosphorescence.

With respiration is necessarily associated in plants a liberation of heat. The proper temperature of plants may under certain circumstances reach a considerable height. It has been found, for example, that actively respiring tissue masses are sometimes warmer by several degrees than their surroundings. In particular the spontaneous development of heat in the spathe of the inflorescences of Aroids is very considerable;[1] but since such material is not always available, we shall first employ germinating seeds to prove that plants produce heat. Experiments with seeds can conveniently be made at any time. We use the apparatus shown in Fig. 107. Under a bell-glass stands a vessel, *G*, containing strong potash solution. In the funnel *T* is first placed a small

perforated filter, and then the seedlings which we wish to examine with reference to their spontaneous development of heat. The tubulus of the bell-glass is fitted with a cork through which passes the thermometer *Tm*, fixed so that the germinating seeds completely surround its bulb. The seedling material is easily obtained by laying seeds (Pisum, Triticum), after soaking, on damp blotting-paper, when germination at once begins. We fit up a second apparatus in exactly the same way, filling the funnel, however, not with developing plants, but with paper balls which have been soaked in water. I experimented, *e.g.*, with wheat seedlings, four days old, placed in a funnel of about 200 c.c. capacity. The thermometer after some time indicated a temperature of 19° C., while the thermometer in the funnel containing moistened paper pellets only registered 17° C. The seedlings were thus 2° C. higher in temperature. Using the above form of apparatus we need not fear that the material will suffer from want of Oxygen, since it is not air-tight, and the Carbon dioxide produced is absorbed by the potash. On the other hand, it is important to put the apparatus together, and introduce the seedlings and paper pellets respectively, some hours before the thermometer readings are to be taken (*e.g.* in a lecture). It is likewise necessary, before making the experiments, to carefully compare with one another the two thermometers which we use. It is also instructive to experiment with flowers instead of seedlings, *e.g.* flowers of Anthemis or Bellis. The development of heat in these is fairly large.

FIG. 107.—Apparatus for proving the production of heat by plants.

To prove that heat is liberated in the alcoholic fermentation set up by yeast, we simply proceed as follows. We prepare two cylinders *A* and *B*. In *A* we place 300 c.c. of Pasteur's food solution (for its preparation see 18); in *B* 300 c.c. of water. In both fluids is placed a considerable quantity of yeast, and they are then kept at a temperature of about 24° C. When active fermentation has been set up in cylinder *A*, we determine the

temperature of the fluids. It is found that that contained in the cylinder *A* is 1° or 2° C. warmer than the water in the cylinder *B*.

In searching and accurate investigations on the proper temperature of plants we may conveniently use a respiratory vessel such as is indicated in Fig. 97, of about 200 c.c. capacity, and provided with a spiral tube. We require two of them. One we fill with four to five days old wheat or barley seedlings, grown in sawdust, the other with seedlings which have been killed by immersion in boiling water to which some Salicylic acid has been added. The two vessels are connected with one another by means of rubber tubing; they are provided with carefully compared thermometers, completely packed in cotton wool, and placed in a wooden box. This is put in a room with a north aspect, in which the temperature varies little. The vessel containing living plants we put in connection with an aspirator, and lead a slow stream of air saturated with moisture over the research material. The air enters through the spiral tube of the vessel containing the dead seedlings. The observations of temperature made from time to time (say every half-hour) indicate that the fresh material rapidly assumes a temperature of 2°–3° C. higher than the dead material. These last do not develop heat, while the living seedlings undergo a considerable rise of temperature.

We will now pass through the apparatus for about an hour a rapid stream of Hydrogen (the gas is purified by being passed through a wash bottle containing Potassium permanganate and potash solution). This being done, we close the stop-cock *H''* (Fig. 97), and also a stop-cock on the spiral tube of the vessel containing dead material. The vessels, still packed in cotton wool, are left alone for a few hours only. The elevation of temperature in the living seedlings is now, since the respiration is intramolecular, only very slight, about 0°·2–0°·3 C.; it rapidly rises, however, to 2°–3° C. when air is again led through the apparatus.

Observations on the heat developed by seedlings in normal respiration showed me also that it is greater the nearer the temperature at which we are experimenting approaches the optimum (40° C.). This is quite natural, because, with rising temperature, metabolism and respiration in the plant-cells (and therefore also development of heat) are more active than at lower temperatures.

When we supply the apparatus not with seedlings, but with adequate quantities of the spadices of Arum maculatum, the

spathes of which have just opened (one vessel with dead, the other with living spadices), we find the elevation of temperature in normal material to be in intramolecular respiration about 0°·2 C., but in normal respiration 10°–15° C.

Exhaustive researches on the proper temperature of flowers have been carried out by Dutrochet, Hoppe, G. Kraus, and many others. We use for the purpose pot plants of Colocasia cordifolium or of Arum maculatum, put into pots with the balls of earth in the spring, a good time before flowering. The plants are placed in diffused light in a room with a north aspect, and liable to as little variation of temperature as possible. The rise of temperature of the flowers cannot be easily determined till the sexual organs have come to maturity, and the spathes open. It can even be detected by simply placing a sensitive thermometer, *e.g.*, against the spadix.

It is better to experiment as follows. The spadix of an Arum is forced a little out of the spathe, and laid against the cylindrical bulb of a sensitive mercurial thermometer, supported by a suitable stand. The spadix is fixed against the thermometer by means of a thin rubber ring. We may also place the upper end of the spadix in contact with a thermometer, whose mercury reservoir is in the form of a double-walled bell. It is also naturally of importance to determine accurately the temperature of the air in the room. It is found that the structures investigated are warmer by some degrees (Centigrade) than the surrounding air. In observations extending over some time (several days) we find that the difference between the temperature of the air and of the part of the inflorescence is not exactly the same at all times of the day. The difference is usually greatest at a particular time in the afternoon. We find, therefore, what is very remarkable, a daily periodicity in the temperature of the flowers.*

If it is required to prove merely in a general way that in the inflorescences of Aroids a very considerable development of heat takes place, plants of Arum maculatum in flower are cut, and placed with the base in water. After removing the spathes, a few spadices are fastened by means of thin rubber rings round the bulb of a sensitive thermometer with a cylindrical bulb.

* In many cases it is also advisable to keep the plants under large, not perfectly air-tight cases made of zinc and glass, so as to considerably reduce their transpiration.

We get two flasks of about 500 c.c. capacity, *a* and *b*, and fill *a* $\frac{2}{3}$ full of water, and *b* $\frac{2}{3}$ full of a fermenting fluid (beer wort with addition of yeast). Both flasks are fitted with three-holed rubber stoppers, through each of which is passed a thermometer, a leading tube reaching to the bottom of the flask, and an exit tube. The flasks having been packed in wadding, the exit tube of *b* is connected up with an aspirator, *a* and *b* are connected by rubber tubing, and a slow stream of air is bubbled through *a*, and then on to *b*. After a time we find that the fermenting fluid is about 2° C. warmer than the water, and this difference of temperature remains when Hydrogen is led through the fluids. Yeast therefore, in contrast with other plants, produces about the same quantity of heat[2] both in normal and intramolecular respiration.

In many cases it is advisable to determine the proper temperature of the plants, by employing a thermo-electric apparatus. This method may, *e.g.*, be used for investigating shoots, the development of heat in which, generally speaking, is only very trifling. I was, *e.g.*, able to show in this way that living Helianthus shoots, about 15 cm. long, in air saturated with moisture, were about 0°·3 C. higher in temperature than dead shoots. We experiment after Dutrochet's method[3] with the apparatus indicated in Fig. 108.

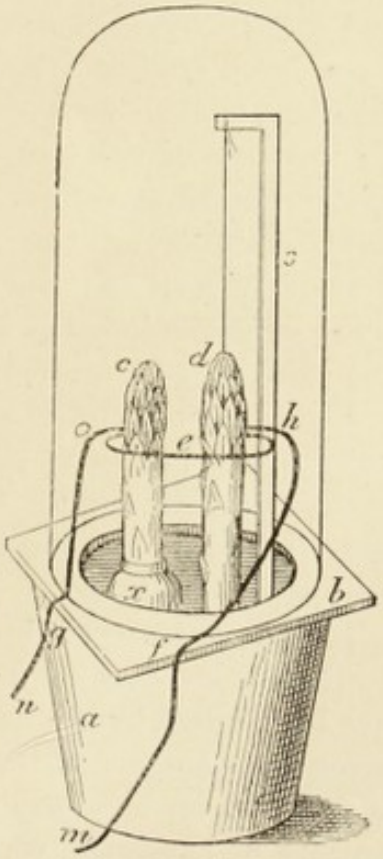

Fig. 108.—Dutrochet's apparatus for thermo-electric researches on plants.

The ends of the iron loop *e* are soldered to the copper wires *o* and *h*, and the junctions, after being carefully varnished, are pushed into the shoots *c* and *d*, of which *c* is living, while *d* has been killed by immersion in hot water. The difference in temperature between the shoots is indicated by the deflection of the galvanometer inserted between the ends of the wires *n* and *m*. The deflection of the galvanometer corresponding with 0°·1 C. difference of temperature,

must of course be determined by special preliminary examination.

In our figure, the dead shoot *d* is fastened by a thread to the support *s*, while the living shoot stands in the vessel of water *x*. To secure the provision of air saturated with moisture, the plants are placed in the flower-pot, *a*, the rim of which supports a sheet of plaster of Paris, on which rests a bell-glass provided round the bottom with moist sand.

In employing the above method, more attention must be paid than hitherto has been to the possibility that electric currents are set up merely in consequence of the contact of the thermo-electric junctions with the moist plant structures.

That some plants emit light in consequence of their vital processes is a demonstrated fact. Thus there are, *e.g.*, a series of phosphorescent bacteria, and I myself once was able to observe the phosphorescence, due to bacteria, emitted from putrid fish.

Similarly the phosphorescence of Agaricus olearius, A. melleus, and Xylaria hypoxylon is well known. The phenomena in question have been studied especially by Fabre and Ludwig in Greiz. Agaricus melleus grows chiefly on conifers, and if in winter we dig up the roots of the trees attacked, or collect portions of the sticks containing its mycelium, we can easily observe in the dark the light emitted by the wood, especially if the material has previously been kept for a day in a damp cellar. If we note the trees or sticks infected by the Agaricus, we can at any time of the year procure luminous wood.

The above-mentioned species of Xylaria is to be found throughout the year on beech sticks. The infected wood shines with a greenish yellow light. The light emitted by "luminous wood" penetrated by Agaricus melleus appears, on the contrary, whitish with a tinge of green.

If luminous wood is immersed for a short time in water at 80–100° C., it loses its power of shining in the dark, since the fungus has been killed by the heat. The luminosity of the wood is also arrested in absence of Oxygen, *e.g.* in a stream of Hydrogen, but is resumed on exposure to the air. The luminosity is due to a process of oxidation, which is dependent, in a manner still not understood, on the vitality of protoplasm.

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[1] See G. Kraus, *Abhandlungen d. naturforsch. Gesellschaft zu Halle*, Bd. 16, In this paper also the literature is collected.

[2] See Eriksson, *Unters. a. d. bot. Inst. zu Tübingen*, Bd. 1.

[3] See Dutrochet, *Annal d. sc. nat.*, 1840, Sér. II. T. 13, p. 5. See also Pfeffer, *Handbuch*, Bd. 2.

III. THE BEHAVIOUR OF NON-NITROGENOUS PLASTIC SUBSTANCES IN PLANTS.

110. Starch as Reserve Material.

In very many receptacles of reserve material, the non-nitrogenous substances are laid down in the form of starch. We may satisfy ourselves of this by mounting in water, and examining under the microscope, delicate sections from the cotyledons of peas or beans, from the endosperm of a wheat grain, from a potato, or from a rhizome of Canna indica. The starch grains in the cells are easily recognised, and for confirmation we may stain them blue by means of Iodine. Even in the medullary rays, and in the wood of trees and shrubs, starch is very generally stored up during the winter as reserve material.[1] I obtained particularly good results with twigs of Berberis vulgaris, Fraxinus excelsior, and Fagus sylvatica gathered in January and February. We prepare transverse and longitudinal sections through the wood of Berberis vulgaris, mount them in a drop of iodised glycerine (prepared by leaving Iodine in glycerine for some time), cover with a cover-glass, and warm the slide over a spirit flame. Microscopic examination after cooling shows that the medullary rays especially, but also the elements of the wood, contain agglutinated masses of starch, which have stained blue. The wood of Fraxinus consists of broad and narrow vessels, scanty wood parenchyma, principally occurring in the neighbourhood of the vessels, and wood fibres. Transverse sections through the wood, treated as indicated with iodised glycerine, show that it is mainly the cells of the medullary rays which are rich in starch. In Fagus there is besides the vessels and wood fibres a fairly large quantity of wood parenchyma, running in tangential bands. This, as also the cells of the broad medullary rays, is very rich in starch. In the spring the starch disappears from the medullary rays and wood of the vascular bundles. Obviously the starch now passes out of these tissues, in which it was stored up as reserve material, and travels to the growing parts of the plants.

To obtain accurate information as to the starch contents of a

rhizome, we select for examination the root-stock of Pteris aquilina, which creeps horizontally in the soil. We may use alcohol material, and select not too thick pieces of the rhizome. The ground tissue consists chiefly of parenchyma, the cells of which contain very large quantities of starch, and is traversed by very strongly developed plates of sclerenchyma, observable even in macroscopical examination of transverse sections of the rhizome as broad, dark lines. Between these plates of sclerenchyma the bicollateral vascular bundles are readily recognised. Each vascular bundle is surrounded by a single layer of cells rich in starch (phloëm sheath, *Vorscheide*) and by the endodermis proper, which is however free from starch.

We shall often recur to the significance of starch stored up in receptacles of reserve material. It need only be mentioned here that starch is the most important non-nitrogenous reserve material in plants, and that when it is to be employed in the development of this or that organ it must first be converted into soluble compounds which can leave the storehouses. Under the influence of diastatic ferments, viz., starch is converted, as we shall see in 112, into glucose, and thus its translocation is rendered possible.

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[1] See Sanio, *Untersuchungen über die im Winter Stärke führenden Zellen des Holzes*, Halle, 1858.

111. The Quantitative Determination of Starch.

Much has already been said elsewhere as to the properties and behaviour of starch. At this place the chief object is to indicate the method to be employed in quantitative determinations of starch. Starch as such is, as is known, unable to reduce Fehling's solution. It can, however, be converted into grape-sugar by the action of acids, and this is readily estimated quantitatively by means of Fehling's solution. 2–3 gr. of pure potato starch, which has been freed from water at a temperature of 100°–110° C., is heated in a flask with 200 c.c. of water. To the fluid is added 20 c.c. of 25 per cent. Hydrochloric acid, and it is then heated for three hours in an actively boiling water-bath, the water lost by evaporation being replaced.* After cooling, the fluid is neutralised

* Sulphuric acid has also been used for converting starch into grape-sugar. Hydrochloric acid, however, is to be preferred. See R. Sachsse, *Phytochemische Unters.*, 1880, p. 47.

with potash, and made up to 500 c.c.[1] We now heat dilute Fehling's solution on the water-bath in an evaporating dish, add 20 c.c. of the sugar solution, and again heat for ten to fifteen minutes.* The Cuprous oxide formed is collected as rapidly as possible on a filter, washed with hot water and dried. We now ignite the filter paper and strongly heat the Cuprous oxide in a platinum crucible with addition of a little Potassium nitrate, and finally determine the weight of the Cupric oxide obtained. 220·5 parts of the Cupric oxide correspond with 100 parts of grape-sugar, or 90 parts of starch. The requisite Fehling's fluid is prepared as follows. We dissolve 34·65 gr. of pure Copper sulphate in 200 c.c. of water, mix with a solution of 173 gr. of Potassium Sodium tartrate in 480 c.c. of caustic soda solution of 1·14 sp. gr. (about 10 per cent. soda solution), and dilute the fluid to 1000 c.c. at 15° C.

It remains to be mentioned that the starch contains very small quantities of mineral matter. These must be determined and allowed for.

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[1] On other, in some respects, still more exact methods of estimating starch, see König, *Anleitung zur Untersuchung landwirthschaftl. wichtiger Stoffe*, 1891, p. 231.

112. The Occurrence of Diastase in Plants, and the Manner in Which the Ferment Acts.

Diastase is of course widely distributed in the vegetable kingdom, but the quantities of diastase present in different kinds of plants is by no means the same. Germinated barley is very rich in diastase. If malt from a brewery is ground up in a small handmill, we obtain a powder which is specially useful for the preparation of a diastase-containing solution. We treat 25 gr. of the malt powder with 100 c.c. of water, stir the mixture frequently, and after some time (*e.g.* one to two hours) filter the solution. If we add 25 c.c. of 1 per cent. starch paste (prepared by mixing 100 c.c. of distilled water with 1 gr. of potato starch, and heating to boiling point) to 5 c.c. of clear diastase solution, a transformation of the starch is quickly observable. Immediately after mixing the starch and malt extract, a sample taken from the fluid assumes a blue

* It must further be noted that the sugar-containing fluids, which are added to the hot Fehling's solution, must not contain more than, say, $\frac{1}{4}$–$\frac{1}{2}$ per cent. of sugar.

colour on addition of a trace of alcoholic Iodine solution. After a few minutes the fluid containing the starch and diastase has become clear, but a sample of it still gives a blue coloration on addition of Iodine. If we wait for some time, a sample of the fluid assumes a violet coloration with Iodine. A sample taken still later becomes brown on addition of Iodine, and finally (perhaps after two or three hours) Iodine no longer produces a marked coloration in a sample of our fluid. Under the influence of diastase the amylum as is known splits up into a series of related dextrins and sugar (maltose). These dextrins do not all assume the same colour on addition of Iodine, and thus the Iodine reaction affords a very convenient means of following accurately the transformation under the influence of diastase. To determine with certainty the diastatic action of a plant extract, especially if the quantity of ferment is inconsiderable, it is necessary for reasons given by Wortmann, in his treatise cited below, not to add the Iodine till the mixture of plant extract and starch paste has been boiled and completely cooled again. If then no blue coloration is found, starch is certainly no longer present. The formation of sugar may also be easily demonstrated. We determine by means of Fehling's solution the amount of sugar present in 5 c.c. of malt extract (see 115), treat 25 c.c. of starch paste with 5 c.c. of malt extract, and after a few hours estimate the amount of sugar in the fluid. It is found to contain far more sugar than did the 5 c.c. of malt extract.

If a not too small quantity of malt extract, made as strong as possible, is treated with a large excess of absolute alcohol, a voluminous precipitate is thrown down. We collect this on a filter, wash it with alcohol, and dry the residue in the air. It consists of a number of different substances, and among them the diastase precipitated by the alcohol. If we dissolve a small quantity of the dry mass in water, we shall obtain a fluid which transforms starch very energetically.

It is of interest to make a few experiments to show that not only barley seedlings, but other seedlings also, and leaves and stems of different plants contain diastase. I have, *e.g.*, experimented with wheat seedlings a few days old, and pea seedlings ten days old (the seedlings had developed in darkness), and also with leaves of Sedum maximum and stems of Impatiens Balsamina. The material was pounded in a mortar, flooded with a little water, and after some time filtered. The solutions obtained transformed

starch, as was shown by means of the Iodine reaction. Since, however, such material does not contain nearly so much diastase as barley seedlings, it is advisable to use very dilute starch paste, and add only very small quantities of it (perhaps 2 c.c.) to a fairly large quantity of the plant extract.[1 2]

To prepare the diastase-containing extracts, the plant structures are cut up, pounded with a little water in a mortar, and placed in from two to four times their volume of water for two to six hours. We then filter. Proceeding in this way we can certainly discover the ferment in plant structures if it is present in good quantity. The method is not, however, suitable for small quantities of diastase, and hence Wortmann's conclusion that many kinds of foliage leaves, for example, in which he could detect no diastase by that method of treatment, contain no ferment capable of isolation. The solution of the starch would then be brought about directly by the protoplasm. This may be true in some cases; Brown and Morris, however, have recently pointed out that in many cases the treatment of fresh plant structures with water for extraction of diastase is not to be recommended at all. It is not easy to crush all the cells of these fresh structures, so that the extraction is incomplete, and in testing for diastase a negative result may be obtained, although the ferment actually occurs in the structures. We obtain much more trustworthy results if we first dry the material at about 40° C., then rub it down very finely, and now add the powder obtained, or an extract of it, to starch paste. It is found in this way that even many foliage leaves are very rich in diastase, as I satisfied myself in the case of foliage leaves of Pisum sativum.[3]

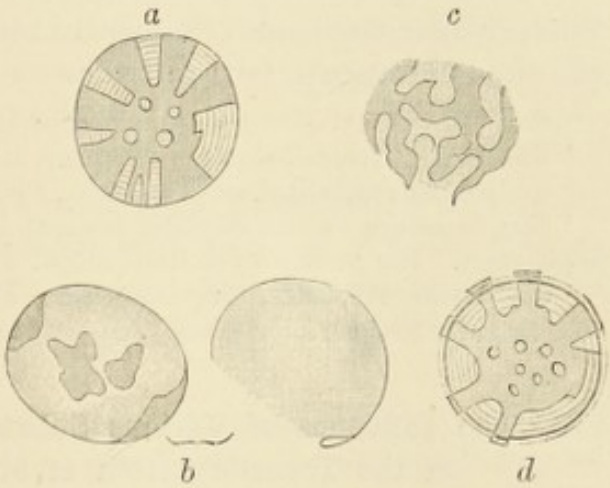

FIG. 109.—Starch grains from the endosperm of a wheat grain in different stages of corrosion. *a*, slightly corroded; *b*, more deeply corroded; *c*, more deeply still; *d*, most strongly of all. (After Baranetzky.)

With reference to the action of diastase in plants, it is very important to satisfy oneself that the ferment can act upon and dissolve not only starch paste, but even uninjured starch grains. To

3 cgr. of air-dry wheat starch in a watch-glass are added 3 c.c. of concentrated malt extract, or 3 c.c. of an aqueous solution of the ferment precipitated by alcohol. If we employ the latter we add to it a very small quantity of Citric acid, since, as is shown in 113, the presence of acid is very favourable to the activity of diastase. The watch-glass is carefully covered, and we observe repeatedly during twenty-four to forty-eight hours the changes which comparatively large starch grains undergo, drops of the fluid being placed on the slide and examined microscopically. I satisfied myself that there was a considerable difference in the behaviour of the starch grains. Generally the corrosions produced have the appearance represented in Fig. 109, *a*, *b*, *c*, and *d*. The solution of the starch substance proceeds from the outside inwards, and it forms bright radiating bands which become broader with increasing corrosion, and gradually the changes proceed further and further inwards, the corrosion canals also frequently becoming branched, so that at last the starch grain falls to pieces.[4]

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[1] See Detmer, *Landwirthschl. Jahrbücher*, Bd. 10.

[2] For further particulars see Wortmann, *Botan. Zeitung*, 1890.

[3] The literature on the diastatic ferment has been very fully discussed by Schleichert. See *Nova Acta d. Leop.-Carol. Academ.*, Bd. 62.

[4] See Baranetzky, *Die stärkeumbildenden Fermente in den Pflanzen*, 1878, p. 48. See also Krabbe, *Jahrbücher f. wissenschl. Botanik*, Bd. 21.

113. The Influence of Various Substances, and of Temperature on the Transformation of Starch by Diastase.

In each of a number of small glasses we place 25 c.c. of 1 per cent. starch paste. The vessel *a* contains at first nothing further; to *b* are added a few drops of Hydrochloric acid; to *c* a few drops of a concentrated solution of Citric acid; to *d* a few drops of potash solution; to *e* a few drops of alcohol; to *f* a few drops of chloroform. Into each glass we now pour further 5 c.c. of malt extract, and at the end of twenty-four hours add to each of the fluids, by means of a glass rod, a small quantity of alcoholic Iodine solution. The fluids *a*, *e*, and *f* do not colour blue. All the rest take on a blue coloration on the addition of the Iodine. Alcohol and chloroform have not arrested the action of the diastase; the acids and the potash, on the other hand, have rendered the ferment inoperative.

As regards the influence of acids in diastatic fermentation, it is,

however, to be emphasised that only large proportions of acid annul the action of diastase. If, on the one hand, we treat 25 c.c. of paste with 5 c.c. of malt extract, and, on the other hand, 25 c.c. of paste with 5 c.c. of malt extract and 2–3 mg. of Citric acid, the transformation of the starch takes place more rapidly in the latter fluid than in the former. Small quantities of Citric acid, therefore (and small quantities of other acids behave in a similar manner), do not arrest the action of diastase, but on the contrary favour it.

If, on the one hand, we mix 25 c.c. of starch paste at a temperature of 15 or 20° C. with 5 c.c. of malt extract at a temperature of 15 or 20° C. and keep the temperature of the mixture at 15 or 20° C., and if, on the other hand, we mix together 25 c.c. of paste and 5 c.c. of malt extract after cooling to 4° C., we can readily satisfy ourselves by means of the Iodine reaction, that the transformation of the starch by the diastase proceeds far more rapidly at the higher than at the lower temperature. The optimum temperature for diastatic action lies at 63° C. (Kjeldahl).

If we heat malt extract to the boiling point, and mix the fluid, after it has been allowed to cool, with paste, it is found that no transformation of the starch ensues. The ferment has been destroyed by the heat.[1]

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[1] See Detmer, *Pflanzenphysiologische Untersuchungen über Fermentbildung und fermentative Processe*, Jena, 1884; also *Landwirthschaftl. Jahrbücher*, Bd. 10.

114. The Production of Diastase in the Cells of Higher Plants.

Into each of two retort-like vessels of about 90 c.c. capacity, we introduce twenty air-dry wheat grains, fill the vessels with water which has been boiled and then allowed to cool again, close the mouths of the vessels with the finger, and invert each of them in the manner indicated in Fig. 11, p. 34, in a beaker containing mercury and water. At the end of twenty-four hours the water in one of the vessels is replaced by atmospheric air, that in the other by Hydrogen. We prepare the Hydrogen by the action of arsenic-free Zinc on dilute Hydrochloric acid in a suitable apparatus, and we pass the gas first through a solution of caustic potash, then through a solution of Potassium permanganate in

order to remove any traces of sulphuretted Hydrogen and hydrocarbons which may be present. A small quantity of water must be left above the mercury in the tubes of the retorts in order to prevent the research material from being exposed to the vapour of the mercury. The wheat grains lying in atmospheric air quickly germinate; those in Hydrogen do not germinate. Control experiments, however, show that the grains exposed to the Hydrogen by no means rapidly perish, but remain capable of germination for a fairly long time (for several days at least), and if they are subsequently exposed to conditions favourable to germination, in presence of air, their embryos will develop. When the material under investigation has been exposed to atmospheric air and Hydrogen respectively for two or three days, it is removed from the vessels, crushed in the mortar, and the pulp is treated with 20 c.c. of water. After some time we filter through filter paper not previously moistened. If we now mix 10 c.c. of the filtrate with 20 c.c. of dilute starch paste, the Iodine reaction shows that the extract from the seedlings developed in the vessel containing air acts energetically on the starch, while the extract from the material kept in Hydrogen exhibits only a very slight power of transforming starch. It is not greater than that of an extract prepared by treating twenty dormant wheat grains, after pounding in a mortar, with 20 c.c. of water. The experiment teaches, therefore, that diastase can only be formed in the cells of higher plants in presence of free atmospheric Oxygen.[1]

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[1] See Detmer, *Botan. Zeitung*, 1883, No. 37, and *Pflanzenphysiologische Untersuchungen über Fermentbildung und fermentative Processe*, Jena, 1884.

115. The Estimation and Microchemical Detection of Glucose.

Dextrose, maltose, etc., are capable of directly reducing Fehling's solution. The sugars which behave in this manner are grouped together under the general name of "glucoses." If it is desired to estimate the amount of glucose in a sample of malt, we first grind the material to powder in a hand-mill, and determine the weight of dry substance in a small quantity of the powder. A further quantity of the powder, say 3 gr., is now treated repeatedly with cold water, and the resulting solution is filtered. Extracts prepared from seeds or seedlings, with which also we are here concerned, frequently do not at once give a clear filtrate.

The filtrate is easily cleared, however, by passing into it for some time washed Carbon dioxide. The combined filtrates are precipitated with Lead acetate, filtered and made up to a known volume, say 200 c.c. In the resulting fluid we determine the sugar by Fehling's solution (see 111).

It is not to be forgotten in working out the results that 100 parts of maltose, which is the sugar especially found in malt extracts, only decompose as much Copper oxide as 61 parts of dextrose (Brown and Heron).

To determine microscopically the presence of glucose in tissues, we first cut sections of the objects, *e.g.* from pears or apples, which, however, must not be too thin, so that not all the cells are opened. It is best for the sections to include three layers of uninjured cells. The sections are placed in a concentrated solution of Copper sulphate at the ordinary room temperature, removed with the forceps after a short time, and washed superficially by dipping in clean water. We now at once place the sections in boiling potash solution,[1] or better[2] in a boiling solution of 10 gr. of Sodium Potassium tartrate, and 10 gr. of caustic potash in 10 gr. of water. If glucose is present, a beautiful red precipitate of Cuprous oxide is produced after a few seconds in the cells which contain it. Microscopical examination of the sections gives information concerning the distribution of the sugar in the tissue.

The following method for detecting glucose is very convenient. The sections are rinsed with water, laid on a slide in a drop of Fehling's solution (see 111), and covered with a cover-glass. We now heat till small bubbles appear, but no longer. In presence of sugar, Cuprous oxide separates out in the cells.

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[1] See Sachs, Pringsheim's *Jahrbücher*, Bd. 3, p. 187.

[2] See Arthur Meyer, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 3, p. 332.

116. Dextrin.

One hundred c.c. of water are mixed with 1 gr. of potato starch, and heated to boiling. To the cooled paste are added a few drops of Sulphuric or Hydrochloric acid, and it is then again warmed. The fluid rapidly clears, and a small sample of it, after it has been allowed to cool, still gives a blue coloration with Iodine. If we continue to boil the acidified solution, remove from it from time to time (say every five minutes) small samples, and treat them

with Iodine solution after cooling, it is found that the first samples on addition of the Iodine become violet, later ones reddish brown, and still later one yellowish in colour. These colour reactions show that under the influence of the acid, different kinds of dextrins are formed from the starch in succession. First, the starch substance breaks down into sugar and amylodextrin I., which gives a violet reaction with Iodine. This dextrin is then split up by the acid into sugar and amylodextrin II., which becomes reddish-brown on addition of Iodine. Still other kinds of dextrin, colouring yellowish with Iodine, are formed together with sugar from amylodextrin II., and finally the dextrins completely disappear, having been entirely converted into sugar.[1] According to recent researches the process is perhaps otherwise.

That dextrins (and especially those which react brownish with Iodine) occur in the cells of plants, we can determine in the following manner. Pea seeds are ground to powder in a hand-mill. We treat with a moderate quantity of water, and after an hour pass pure Carbon dioxide into the turbid fluid. We then filter, this operation being very much facilitated by the presence of the Carbon dioxide. A small quantity of the clear filtrate is next brought into contact with a crystal of Iodine, and we shall find that the fluid by degrees assumes a brownish colour; it behaves like an aqueous solution of commercial dextrin placed in contact with Iodine. Pure water assumes in contact with solid Iodine only a yellow tint.[2] If we investigate the behaviour of the aqueous extract of pea seeds towards Fehling's solution, it is seen that no reduction takes place. If, on the other hand, we boil an aqueous extract for some time after adding a few drops of Sulphuric acid, the fluid will be in a position to reduce Fehling's solution energetically, since the dextrin under the influence of the acid has been converted into glucose.

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[1] See W. Naegeli, *Beiträge zur näheren Kenntniss der Stärkegruppe*, 1879, and Detmer, *Landwirthschaftl. Jahrbücher*, Bd. 10, p. 752.

[2] See Detmer, *Journal f. Landwirthschaft*, 27. Jahrgang, p. 379.

117. Estimation of and Microchemical Tests for Cane-Sugar.

Cane-sugar is a constituent of the sap of many plants, and the sap of the beetroot contains an especially large proportion of it. To determine the quantity of sugar present in the roots, we follow

E. v. Wolff's[1] method. The carefully cleaned roots are cut into slices, and 500–1000 gr. of these slices are hung up by threads in the drying chamber at a temperature of 60°–70° C. The dry mass is pounded to a not too fine powder and weighed, and then the amount of dry substance in a small quantity of it (5–6 gr.) is determined. A quantity of the powder (2–3 gr.) is repeatedly boiled with 80–85 per cent. alcohol, the solution being filtered after each boiling, and finally the residue on the filter is washed with hot alcohol. To the whole solution we now add a large quantity of water, and warm on the water-bath till the alcohol has completely evaporated. The fluid is now made up to 300 c.c. In 100 c.c. of it we at once determine the grape-sugar with Fehling's solution; it must, however, be remembered that the grape-sugar is very small in quantity, if not entirely absent. To 200 c.c. of the fluid we add four drops of Sulphuric acid, and warm on the water-bath for three hours, adding water to replace that lost in the process. We then make up to 400 c.c., and after neutralising with Sodium carbonate determine the amount of grape-sugar present in 100 c.c. by means of Fehling's solution. From the numbers obtained it is easy, finally, to calculate the amount of cane-sugar in fresh roots, or in dry root substance. As regards the preparation and method of using Fehling's solution, all that is requisite has been already given (see 111).

From what has been said, it is at once clear how we must proceed to detect qualitatively the presence of cane-sugar in roots.

To detect cane-sugar in roots microchemically, the sections, which must not be too thin, so that all the cells are not opened, are treated as described in 115 with Copper solution and potash. On examining the sections under the microscope, it is found that the contents of their cells has taken on a beautiful blue colour which indicates the presence of cane-sugar.[2] The reaction takes place when the sections are mounted in a drop of Fehling's solution, covered with a cover-glass, and warmed (see 115).

[1] See E. von Wolff, *Anleitung zur chem. Unters. landwirthschl. wichtiger Stoffe*, 1875, p. 184.

[2] See Sachs, Pringsheim's *Jahrbücher*, Bd. 3, p. 183.

118. Reserve Cellulose and Amyloid.

In the seeds of many plants cellulose is present as nitrogenous reserve substance. A date stone is halved transversely, and then

a delicate transverse section of the endosperm is prepared from it by means of a very sharp razor. The walls of the elongated cells are exceedingly thick, but numerous simple pits are present. We treat a section with iodised Potassium iodide solution, and then run in from the margin of the cover-glass dilute Sulphuric acid, prepared by mixing 2 parts by volume of Sulphuric acid with 1 part by volume of water. The thickening layers of the cell-wall stain beautifully blue. If sections of the endosperm are treated in the manner previously described (see 42) with phloroglucin solution, and then with Hydrochloric acid, the walls do not take on a red coloration. The thickening layers of the cell-walls of the endosperm cells are therefore not lignified; they consist of cellulose. In the germination of the date seed this cellulose is made use of.

The material is not, however, absolutely identical with ordinary cellulose, as recent investigations, *e.g.* those of Reiss (*Landwirthschaftl. Jahrb.*, 1889) have shown, and it is hence distinguished as "reserve cellulose."

In the cells of the cotyledons of dormant seeds of Tropæolum majus are present, as is easily seen in examination of transverse sections, parenchymatous elements which are provided with strongly thickened pitted membranes. Between the cells are three-cornered intercellular spaces. The thickening layers stain blue on direct treatment with dilute iodised Potassium iodide solution; they consist not of reserve cellulose but of amyloid. This amyloid is made use of when the seeds germinate. It is dissolved with formation of corrosion canals, and finally only the middle lamella between adjoining cells of the parenchyma is left.

119. Inulin.

Inulin is particularly abundant in the underground organs of many Compositæ. It occurs in solution in the cell-sap, and functions as non-nitrogenous reserve material. If some inulin is treated with cold water, it is found to dissolve with some difficulty. If, however, we apply heat, the inulin completely dissolves. Inulin is unable to reduce Fehling's solution. If, however, we treat hot Fehling's solution with a solution of inulin prepared by heating, a separation of small quantities of Cuprous oxide does take place, owing to the fact that hot water of itself is able to convert small

quantities of the inulin into glucose. If we boil an aqueous solution of inulin after addition of a few drops of Sulphuric acid, a considerable quantity of glucose is produced, and the fluid now reduces Fehling's solution very energetically.

It is noteworthy that if a hot solution of inulin be allowed to cool, the inulin does not at once separate out, but only after some time. If, however, to a solution of inulin which has just cooled we add a large excess of alcohol, the separation of the inulin quickly follows. The insolubility of inulin in alcohol is utilised in testing for the substance microchemically. Sections, not too thin, are cut from the pith of tubers of Dahlia variabilis, covered with alcohol, and after some time dipped in water. On examining the sections under the microscope in water, the inulin is seen to have separated out. Under some circumstances the inulin separates out in the cells in the form of sphere crystals. These are very clearly seen, if we allow fairly large pieces of Dahlia tubers to remain in spirit for at least eight to fourteen days, and then prepare sections from the alcohol material, and examine them in water. The sphere crystals are seated on the cell-walls as globular structures of characteristic appearance [1] (see Fig. 110).

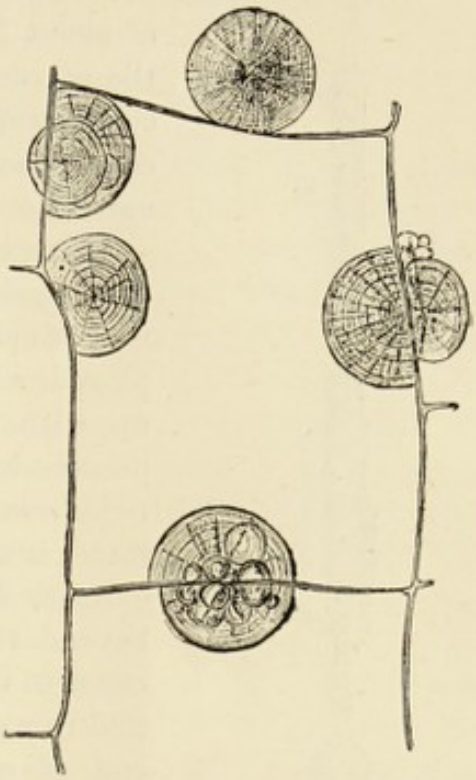

FIG. 110.—Cell from a piece of a tuber of Dahlia variabilis, which had been kept for several months in alcohol. Sphæro-crystals on the walls. Magn. 240. (After Strasburger.)

[1] See Sachs, *Botanische Zeitung*, 1864, p. 25, and Prantl, *Das Inulin*, München, 1870.

120. Vegetable Fats and their Quantitative Determination.

If we extract dried and pounded plant material with ether, we obtain a solution which, on evaporation, yields a residue consisting essentially of fat. Usually the quantity of other substances mixed

with the fat is so small, that in the quantitative determination of fats in plant structures it is generally unnecessary to notice these impurities. In determinations of fat it is convenient to use the apparatus depicted in Fig. 111.

The apparatus, constructed as described by Soxhlet, is to be obtained from Muencke, in Berlin, at a price of about 10 m. It consists of the flask *K*, the extraction arrangement proper *E*, and the condensing arrangement *Kv*, which is put in communication with the water supply. For extraction we employ anhydrous ether.

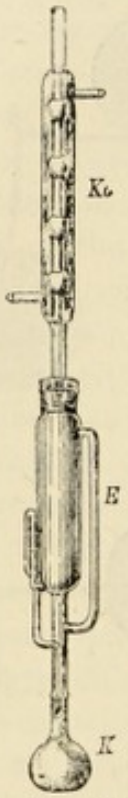

Fig. 111.—Apparatus for fat extraction.

To make the experiments we place in a case made of blotting-paper 3–5 gr. of the very finely powdered substance to be employed, which, if rich in fat, it is best to rub up with clean quartz sand. The case we prepare by rolling a piece of blotting-paper twice round a cylinder of wood, whose diameter is 4 mm. less than that of the extraction cylinder *E*, letting the blotting-paper project beyond the base of the wood to a distance equal to the diameter of the latter. We then fold this projecting part as in making up the end of a parcel, and level it by pressing strongly. After filling in the substance, we similarly close the upper end of the case.

The filled case is now put for two to three hours into a drying chamber at a temperature of 45° C. Then follows the extraction in the usual manner with ether. Finally the ether is distilled off from the solution of fat in the flask *K*, and the residue (crude fat) is dried for one to two hours at 95° C., and weighed.

The fats consist of a mixture of free fatty acids and glycerides. To detect glycerine in these last I proceeded as follows:—About 75 c.c. of olive oil were digested for a considerable time with dilute potash solution in a porcelain evaporating dish on the water-bath. After cooling, a large quantity of Sodium sulphate was added to the fluid, the precipitated soap filtered off, and the filtrate neutralised with Sulphuric acid. The fluid was then evaporated, the residue treated with alcohol, the separated sulphates filtered off, and the filtrate again evaporated. The residue was once more treated with alcohol to purify it, and the solution after filtration

again evaporated. There remains behind a syrupy fluid of sweetish taste, which is glycerine. If this residue is dissolved in water, and a part of the fluid mixed with a dilute solution of Copper sulphate, to which potash solution has been added, so that it holds in suspension a precipitate of Copper hydrate, then the Copper hydrate will be dissolved (glycerine reaction).

121. Reactions of Fatty Oils.

With a glass rod we place a drop of any fatty oil on a glass slide, add to it a mixture of alcohol and ether, which dissolves the fat, cover with a cover-glass, and observe under the microscope. When the alcohol and ether have evaporated, we shall see in our preparation large and small drops which consist of fat. In optical section these appear light grey, and are bounded by a narrow black ring. If the tube of the microscope is lowered, each oil drop will appear bounded no longer by a black ring, but by a bright one. The oil drops cannot well be confused with bubbles of air, for if we focus these under the microscope, and lower the tube, their margin does not become bright, but, on the contrary, the dark band already present increases in breadth.

In the cells of the endosperm of Ricinus, or in those of the cotyledons of Brassica, there are present, besides the proteids, large quantities of fat, and to prove this we need only treat thin sections of the seeds on the slide with a mixture of alcohol and ether. The drops of fat at once separating out are readily recognised as such.

Tincture of alkanna also (a deeply coloured extract prepared by treating alkanna root with 70 to 80 per cent. alcohol) may be used for the detection of fats. If we examine, *e.g.*, sections from the endosperm of Ricinus, whose cells contain a fat differing from others in being soluble in alcohol, we treat the alkanna tincture with an equal volume of glycerine, pass the sections a few times to and fro in the mixture, wash in alcohol, and mount in glycerine. The aleurone grains are stained lightly or not at all, while the ground-mass, in consequence of its fatty contents, is stained deeply red.

A further reagent for fatty oils is an aqueous 1 per cent. solution of Osmic acid. If we lay sections from the endosperm of Ricinus in the solution, they assume after some time a dark colour owing to the blackening of the fats by the Osmic acid. It is to be

observed, however, that fats are not the only substances stained by alkanna tincture and Osmic acid.

122. The Behaviour of Fats in the Germination of Seeds.

Very many seeds (Ricinus, Helianthus, Cucurbita, Brassica, etc.) contain fat as non-nitrogenous reserve material. This fat is physiologically equivalent to the starch of starchy seeds. It furnishes the material for respiration, and likewise the material for the formation of the cell-walls. In many cases, however (*e.g.* Ricinus, Cucurbita), before reaching the place of employment, it is first converted into starch and sugar. In other cases, *e.g.* in Linum, this can hardly be observed at all, and then the fat must travel in the seedlings mainly as such, in order to render possible the translocation of non-nitrogenous plastic material. In fact, such a migration of fat from cell to cell has been observed by H. Schmidt. We will here follow closely the changes which take place during the germination of a fatty seed, where this is associated with the formation of large quantities of carbohydrate.

We select for examination seeds of Ricinus communis. The embryo occupies a central cavity in the copiously developed endosperm, and consists of an axis bearing two thin cotyledons. The large cells of the endosperm contain, as we have already determined in another connection, a matrix rich in fat and proteids, in which lie the aleurone grains. Starch is not present in the cells of the endosperm, or in those of the embryo, as we can readily determine by means of Iodine reagents. A few Ricinus seeds are germinated in garden earth in a flower-pot, in the dark, and at a not too low temperature (about 20° C.). When the main root and the hypocotyl have elongated considerably, the upper part of the latter organ being, however, still curved owing to the fact that the cotyledons are still embedded in the endosperm, the cells of the endosperm, as before germination, contain no starch, but only proteid and fat. The function of the cotyledons is to absorb the reserve materials from the endosperm, so that they can be made use of by the young seedling. Much fat is present in the cells of the parenchyma of the cotyledons; starch, which, as mentioned, was completely absent from the cotyledonary tissue before the commencement of germination, is found abundantly in those parenchyma cells which surround the midribs on the outside. The cambium cells of the hypocotyl contain only proteid material.

The parenchyma of the cortex and of the pith of the upper not yet fully elongated part of the hypocotyl is, as can readily be determined in the usual manner, very rich in starch and sugar, while the quantity of these substances in the lower elongated part

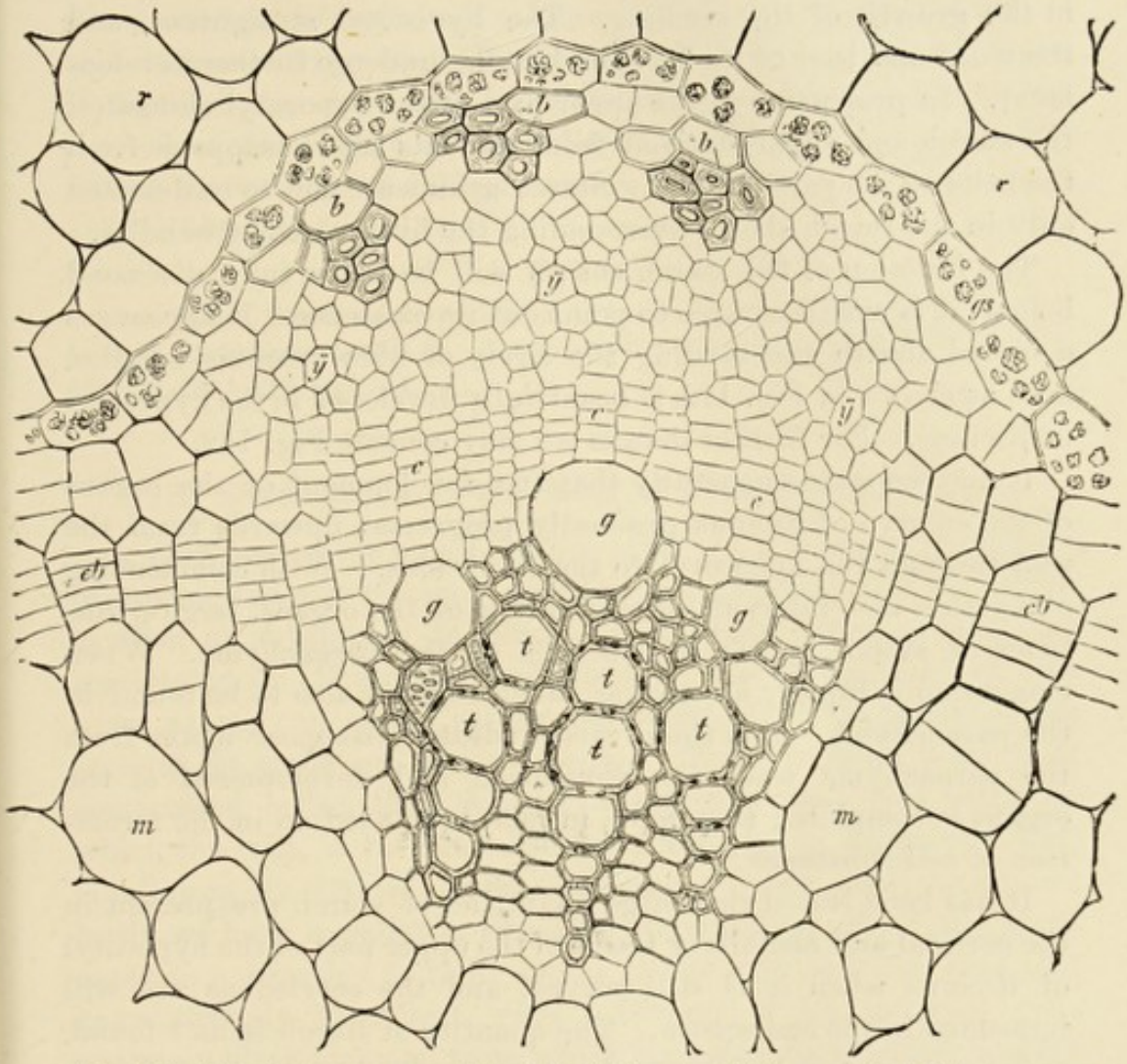

FIG. 112.—Part of a transverse section of the fully extended hypocotyl of Ricinus communis. The longitudinal section is represented in Fig. 33. *r*, parenchyma of the primary cortex; *m*, the pith. Between *r* and *b* the single-layered vascular bundle sheath with starch grains (starch sheath). The fibro-vascular bundle consists of the phloem, *b*, *y*, the xylem, *t*, *g*, and the cambium, *c c*. The cambium, *c c*, is continued laterally in the ground tissue between the neighbouring bundles as interfascicular cambium, *cb*. In the phloem are *b b*, bast fibres; *y y*, partly parenchyma, partly sieve tubes; in the xylem are *t t*, narrow pitted vessels; *g g*, wide pitted vessels, and between them wood fibres. (After Sachs.)

of the hypocotyl more and more diminishes. In the fully elongated part of the hypocotyl, sugar and starch are wanting in the parenchyma; starch grains are only to be detected in the cells of the starch sheath surrounding the ring of vascular bundles. In the tissue of the main root neither starch nor fat are now to be found,

but the parenchyma of the active elongating secondary roots contain much sugar.

As the germination proceeds, the fat keeps disappearing more and more completely from the endosperm as it finds employment in the growth of the seedling. The hypocotyl straightens, and the wood and bast of its vascular bundles undergo further development. In proportion as the upper part of the hypocotyl elongates, the starch and sugar derived from the fats also disappear from the cells of the parenchyma. Starch grains are now to be detected only in the starch sheath surrounding the fibro-vascular bundles.

The function of the starch sheath will be more fully discussed below; it is sufficient here to point out its existence. It appears as a closed sheath surrounding the circle of fibro-vascular bundles, and, as shown in Fig. 112, is beautifully developed in the hypocotyl of Ricinus. For further details we may consult Fig. 112.

It may be stated generally that the development of the organs of the embryo of Ricinus gradually progresses upwards from the root through the hypocotyl to the cotyledons. With commencing elongation and internal differentiation of the organs, large quantities of starch appear in the cells of the parenchyma. When elongation is rapidly advancing, much sugar is also to be found in the parenchyma. But these carbohydrates disappear again from the parenchyma when the elongation and development of the organs is complete; they have, in fact, been used up in the formation of cell substance.[1]

It has been stated that large quantities of starch are present in the cortical and medullary tissue of the upper part of the hypocotyl of Ricinus when it is still curved, and the cotyledons are still imbedded in the endosperm. The quantity of starch is, as I found, so large, that it is possible to demonstrate its presence macroscopically in lecture. For this purpose sections from the upper part of the hypocotyl are treated on a slide with chloral hydrate, and iodised Potassium iodide solution; if the students hold the preparation, covered by a cover-glass, to the light they can satisfy themselves of the presence of large quantities of starch by the blue coloration produced.

In the germination of seeds of Raphanus sativus also a fair quantity of starch appears. I found large quantities of starch in the elongating hypocotyl of a seedling of this plant about four days old, and grown at a temperature of 20° C. The root of the seedling was very poor in starch. In the hypocotyl I found the

starch chiefly in the neighbourhood of the vascular bundles, but also in the rest of the parenchyma. When much starch appears in the seedlings from oily seeds, the cotyledons also, which in the dormant seed are free from starch, for the most part contain at the commencement of germination large or smaller quantities of starch, and the non-nitrogenous bodies travel in the form of carbohydrate. If, however, fat migration is exhibited (*e.g.* in Linum), then the cotyledons are always devoid of starch, and in the other organs also of the seedlings carbohydrates are for the most part present in only trifling quantity.

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[1] See Sachs, *Botan. Zeitung*, 1859, p. 177; also Detmer, *Vergleichende Physiologie des Keimungsprocesses der Samen*, 1880, p. 316; and H. Schmidt, *Flora*, 1891, pp. 320, 342, and 344.

123. Germination of the Seeds of Phaseolus multiflorus.

A very favourable object for the study of a series of metabolic processes, and also of many phenomena connected with the translocation of substances in plants, is the germinating seed of the scarlet-runner (Phaseolus multiflorus).[1] The seed-coat of the bean, as we can easily satisfy ourselves by studying transverse sections, is made up of four layers. The innermost consists of compressed cells; then follows a layer, several cells thick, of which the cells, in variegated seeds, contain a red pigment. This is followed by a third layer, consisting of very small cells; and lastly we have a palisade layer, the elements of which are elongated in a direction at right angles to the surface of the seed, and have strongly thickened walls. Groups of the palisade cells contain a black pigment, from which the seeds derive their spotted appearance. To obtain good preparations from the seed-coat it is, in my experience, advisable to first soak the beans for twenty-four hours, and then let them dry for twelve hours. Material prepared in this manner serves well for the preparation of thin transverse sections of the seed-coat. The seed-coat surrounds the embryo, which consists of two cotyledons and the axis (root, hypocotyl, first stem internode, and terminal bud), with the two primordial leaves. We easily satisfy ourselves that the cotyledons, being concave on their inner sides, leave between them a cavity, and that the axis of the embryo is bent to form an angle.

The cotyledons are made up of the epidermis, the largely

developed parenchyma, and the vascular bundles traversing it. The epidermal cells contain no starch; the elements of the parenchyma, on the contrary, are very rich in starch. Besides starch they contain proteids, as we can easily find by treating sections from the cotyledons with Iodine and Fehling's solution. The cells of the vascular bundles are free from starch, but contain proteids. Delicate transverse sections through the axis of the embryo show that this is made up of an epidermis, cortical and medullary parenchyma, and the intermediate vascular bundle region. The primordial leaves possess stalk and lamina. If a leaf is spread out on a slide in a drop of water, covered with a cover-glass, and submitted to microscopic examination, nerves are seen to be present in the lamina. All the cells of the parenchyma of the lamina are free from starch, but contain proteids.

If bean seeds are laid in damp soil, germination at once begins. The young plant grows at the expense of the reserve materials present in the cotyledons. We notice particularly in studying the developing seedlings the emergence of the root from the seed-coat, which takes place first, the appearance of the stem, bent at the tip, the development of the secondary roots, the formation of the root-hairs and of the hairs on the stem, which are not yet present in the dormant seed, the growth of the primordial leaves, and the differences which are observable between germination in darkness on the one hand, and in light on the other, etc.

As regards the behaviour of plastic substances in germination, the following is specially to be noted. When the root has attained a length of 2–3 cm., many small starch grains are present in the cortex and pith of the root, and of the hypocotyl, while the cells of the axis of the embryo, before the commencement of germination, usually contained but little starch. Glucose occurs in the cells of the cortex and pith of the axis, as we can prove by treating the sections with Copper solution and potash, while proteids are chiefly found in the vascular bundle region. As the root lengthens in the course of germination, and the first internode of the stem undergoes considerable elongation, the starch disappears from the fully elongated cells of the cortex and pith. Thus, *e.g.*, the cells of the cortex and of the pith at the base of the stem soon become free from starch, while the cortex and pith of the upper parts of the stem still contain starch. Ultimately, however, this also disappears. Similarly starch also disappears from the primordial leaves as they develop. When the elon-

gation of the first internode of the stem is completed, its cortex and pith are completely free from starch. Only the very beautifully developed starch sheath, which consists of a single layer of cells, and surrounds the circle of vascular bundles, still contains starch grains in large numbers. The cells of the pith and cortex, which have lost their starch, now contain sugar, but this also disappears more and more as the germination approaches completion. Proteids are specially abundant in the sieve part of the vascular bundle, as may be determined by means of Copper solution and potash. When the primordial leaves are fully developed, the germination stage may be considered at an end. The cotyledons are almost completely free from reserve materials. Sections treated with Copper solution and potash no longer give a violet coloration, since proteids are not present, but stain light blue. The quantity of starch in the cotyledons is now only trifling.

The solution of the starch grains in the cotyledons begins as soon as vital activity is manifested in the embryo, and it is indeed those cells of the cotyledons which lie next to the axis whose grains are first attacked. When the first internode of the seedling is rapidly elongating, there are already large quantities of corroded starch grains present in the cells of the cotyledons together, it is true, with still uninjured ones, and henceforth the process of starch solution keeps spreading.

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[1] See Sachs, *Sitzungsberichte d. Akad. d. Wiss. zu Wien*, 1859, Bd. 37, p. 57, and Detmer, *Vergleichende Physiologie des Keimungsprocesses der Samen*, 1880, p. 308.

124. The Germination of Triticum vulgare.

In order to study carefully the nature of the dormant wheat fruit, we cut sections from grains softened to a certain extent in water. The fruit consists of the pericarp and seed-coat already mentioned elsewhere, and the endosperm and the embryo. We first prepare transverse sections through a grain, and determine that the outermost layer of the endosperm consists of a simple layer of almost square cells, whose membranes are strongly thickened, and which have granular contents. Starch grains are not present in these cells, but they contain large quantities of proteid, as may be easily ascertained by means of Iodine, or Copper

sulphate and potash. The main mass of the endosperm is seen to consist of cells rounded in cross section, which contain starch grains of different sizes, and proteids. The latter are to be detected by treating sections with Copper sulphate and potash solution. The embryo lies at the side of the endosperm. To study the embryo, median longitudinal sections of the wheat grain are to be prepared. These, as seen first under a low power of the microscope, then under a high power, present a complicated appearance (see Fig. 71). Attention must first be directed to the part of the embryo abutting directly on the endosperm. This is the shield (scutellum) which demands our special attention. It mainly consists of small rounded cells; but the layer of cells bordering upon the endosperm is different in character, as can more especially be seen by treating the sections with potash solution. The cells, viz., of this epithelium of the scutellum are elongated and cylindrical in form. At the upper part of the embryo we further recognise the closed sheath leaf, the young rudiments of foliage leaves, and the vegetative cone. The root of the wheat embryo is surrounded by a root sheath (coleorhiza), the boundary between the two being marked by a bright line. Starch or glucose are present neither in the cells of the scutellum, nor in those of the rest of the embryo. On the other hand, all the cells of the embryo contain large quantities of proteid. It is further to be noted that the endosperm of the resting wheat grain also contains no sugar.

Fig. 113.—Seedling of Triticum vulgare.

If soaked wheat grains are placed on plates of pumice-stone lying in water, germination at once begins, the organs of the embryo growing at the expense of the plastic materials conveyed to them from the endosperm. The root emerges, and the first lateral roots, which develop henceforth more rapidly than the primary root, make their appearance. Similarly the primordia of the leaves rapidly undergo considerable elongation, but they still remain at first enclosed by the actively growing first sheath leaf (see Fig.

113). The stem does not develop till later. The seedlings must now be examined from time to time, if it is desired to obtain information respecting the metabolic processes taking place during germination. Soon after germination begins, considerable quantities of glucose appear in the endosperm, as can easily be determined by means of Copper sulphate and potash. The scutellum, which during germination remains within the wheat grain, effects the transfer of the whole of the plastic material from the endosperm into the embryo, but it is important that the cells of the scutellum never contain sugar.

The columnar epithelium serves the scutellum as an absorbing organ, and although the presence of starch or glucose is never to be detected in the epithelial cells, yet the other cells of the scutellum soon after the commencement of germination contain transitory starch. The presence of this starch in the cells of the scutellum may be readily proved by treating the sections with potash, Acetic acid, and dilute Iodine solution. It is further to be noticed here that, in order to prepare satisfactory sections, the germinated wheat grains must be somewhat dried. Proteids, as well as non-nitrogenous bodies, are conveyed from the endosperm to the germinating seedling by the scutellum. If wheat seedlings have developed for, say, five days at the usual room temperature, it is easy, by treatment with Copper sulphate and potash, to detect the presence of considerable quantities of proteid in the younger parts of the roots, and also in the vascular bundles which stand diametrically opposite one another in the sheath leaf. This last is still in a state of very active growth, and is also correspondingly rich in plastic material coming from the endosperm. In the parenchyma of the sheath leaf, numerous starch grains are easily recognised, the number of which diminishes as the growth of the sheath, with progressing germination, gradually ceases. The cells of the rest of the growing leaves also contain starch grains. The presence of glucose I have been unable to detect at any time in any part of the wheat embryo (the seedlings which I examined developed in darkness); glucose is present only in the endosperm of germinating wheat grains. It is, however, by no means impossible that under particular conditions glucose may appear also in the embryo. Naturally the cells of the endosperm constantly become poorer in reserve substances (proteids and starch) as the development of the seedling advances, and if we tease up small quantities of fairly exhausted endosperm tissue in a drop of water

on a slide, and examine with as strong magnification as possible, we shall find, together with uninjured starch grains, others which owing to the action of the diastatic ferment developed in germinating wheat, appear corroded and, as it were, gnawed to pieces.[1]

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[1] See Sachs, *Botan. Zeitung*, 1862.

125. The Germination of Potatoes.

Potatoes consist almost entirely of thin-walled, starch-containing parenchyma. Not only are the medullary and cortical tissues mostly of this character, but also almost the whole tissue of the circularly arranged vascular bundles is parenchymatous in character, and contains starch grains of various sizes. In the wood of the vascular bundles there are present only isolated groups of lignified elements (vessels and wood fibres), while in the bast occur isolated strands whose cells contain not starch but proteid matter. The cortical parenchyma of the potato becomes smaller celled, and poorer in starch, as we pass from the inside towards the skin. On the other hand, the cells of the cortex, close below the skin, frequently contain pigments, etc., dissolved in the cell-sap. The skin of the potato consists of cork tissue, whose cells are tabular in form.

I have often satisfied myself that potatoes placed in autumn in a vessel whose cover is not air-tight, do not germinate for a long time. The potatoes under ordinary conditions undergo a period of rest before germination. This does not begin till about the new year, when single buds of certain eyes, especially those in the part of the potato opposite the old point of attachment to the runner, gradually enlarge and grow. We now leave the potatoes in darkness, and without provision of water. At the beginning of March, many shoots of the potato have already become a few centimetres long, and small scale leaves are visible on them. In transverse sections through the stem of the shoots it is easy to make out the epidermis, the parenchyma of the cortex and pith, and the circle of vascular bundles. Applying the usual microchemical tests to determine the distribution of material in the shoots at different stages of development, we arrive at the following chief results. The parenchyma of very young shoots contains much starch. As the cells of the parenchyma become older, and actively elongate, they become especially rich in glucose. Pro-

teids are found in a state of migration in the soft bast of the bundles. The cambium, the growing points of the stems, and the primordia of the adventitious roots, which originate in the internodes, and which, as I have often found, do not in shoots developing in dry air break through the epidermis, only contain proteids, which is, in fact, in accordance with a general rule that in tissues, *e.g.* the cambium, whose cells are in a very active state of division, carbo-hydrates cannot be detected, because of the extreme rapidity with which they are consumed. I do not here enter into further details, which, however, are easily made out.[1] See also 126.

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[1] See H. de Vries, *Landwirthschl. Jahrbücher*, Bd. 7, p. 217.

126. The Influence of Temperature on the Amount of Sugar in Potatoes.

The study of potatoes as regards the quantity of sugar which they contain presents great interest, since investigations of this kind give results which are of value in examining quite a series of physiological questions. We proceed as follows :—After rubbing down the potatoes (say four) on a grater or by means of a broad file (rasp), to a fine pulp, we place this on a piece of boiled linen lying in a large porcelain dish, and squeeze out the juice with the hand. We rinse the grater or file, and also the hands, with water, and mix the rinsing water with the pressed residue of the pulp, again squeeze, and repeat these operations twice more. The fluid obtained is run into a flask of half a litre capacity. We fill this up to the mark, and treat a certain quantity of the fluid with Lead acetate to precipitate the proteids, etc., filter, and determine the sugar in the filtrate by means of Fehling's solution. Potatoes just matured contain sugar. If we examine ungerminated potatoes which have been kept for some time (a few weeks) in a warm room, at a temperature of 15°–20° C., we find that they do not contain sugar. If such potatoes, devoid of sugar, are placed for about fourteen days in a place (say a cellar) whose temperature does not sink below 0° C., but still does not rise above 2°–3° C., they become sweet, and contain a good deal of sugar. It is very desirable, in investigating the effect of cold, to put the potatoes in a thermostat placed in the cellar. The apparatus consists of a double-walled zinc vessel. The space between the walls is filled

with ice, and instead of having an ordinary lid the apparatus is closed by a zinc tray filled with ice. The potatoes are thus exposed in the thermostat to a constant temperature of 0° C. For further information respecting such a thermostat see 49. If potatoes which contain little or no sugar are placed in a glass vessel, this in a freezing mixture (snow or ice and common salt), so that the potatoes rapidly freeze, and become ringing hard, there is no change in the quantity of sugar present, as is indicated by grating them down while frozen, and extracting with water. These facts were first demonstrated by Müller-Thurgau.[1] A few observations on the subject are also to be found in a short treatise published by me.[2] Müller-Thurgau has found that the relation between the processes of sugar formation and respiration in potatoes is very different at different temperatures, and that it is very important in explaining the phenomena observed to keep this in view. At a high temperature (say 15°–20° C.) respiration proceeds comparatively energetically, so that the sugar is used up as fast as it is formed, and cannot accumulate in the potatoes. At a lower temperature (*e.g.* 0°–3° C.) more sugar is formed from the starch present than can be made use of in the now feeble respiration. Consequently at lower temperatures accumulation of sugar takes place. The mere freezing of potatoes is without influence on the quantity of sugar they contain. For observations on respiration see 102.

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[1] See Müller-Thurgau, *Landwirthschl. Jahrbücher*, Bd. 11, p. 751.

[2] See Detmer, *Pflanzenphysiologische Untersuchungen über Fermentbildung und fermentative Processe*, 1884, p. 41.

127. The Ripening of Fruits and Seeds.

If a thin transverse section from a ripe seed of Brassica Napus is examined, it is seen that the seed-coat is made up of a number of different layers. Passing from the outside inwards we have first a colourless layer consisting of compressed cells, followed by a layer consisting of brown-coloured cells, the lumina of which are fairly distinct. Upon this abuts a layer composed of brown compressed cells, followed by a fourth whose cells are strongly thickened, and exhibit distinct lumina, while the fifth layer, like the first, no longer presents cellular structure. The second and third layers of the seed-coat cause the brown coloration of the seed. In the cells of the folded cotyledons appear large quantities

of proteids and fats; starch is completely absent in the ripe seeds. The still unripe maturing seeds possess, however, much starch. It streams to them from the assimilatory organs of the plant, and is ultimately completely used up for the formation of fat in the seeds. We prepare transverse sections from a green pod of Brassica Napus (I examined, *e.g.*, on May 20th, pods 6–8 cm. in length). Between the two carpels we see the false septum. The fruit tissue itself consists of a strongly cuticularised epidermis, green and colourless ground tissue, and a number of vascular bundles. The young seeds are seated on the united margins of the carpels. If we treat sections with chloral hydrate and iodised Potassium iodide solution, we find that the tissues of the fruit and seed contain much starch.

The ripe hypanthium of the pear is, as is known, very rich in sugar. Since the fruit itself contains but little chlorophyll, it follows that at all events most of the material required for its growth, and for the production of the sugar deposited in the hypanthium tissue, must be conveyed to it from elsewhere. The fruit stalk conducts the plastic material. If we prepare transverse sections from the fruit stalk of the pear (I made an examination on the 8th of June), the circle of vascular bundles between the cortex and the pith can be at once recognised even under low magnification. The bast part of the vascular bundles has on the outside a thick layer of bast fibres, then immediately follows a layer of cells (the starch sheath) in whose elements we observe large quantities of starch. In the parenchyma of the hypanthium of the pear, I found on June 8th but little starch; it was present only in isolated cells, obviously indicating that the starch is very rapidly used up. At a still earlier stage of development (on May 2nd) I could detect no starch in the cells, even on treating thin sections of the hypanthium with chloral hydrate and iodised Potassium iodide solution.

If we cut transverse sections through a flower of Phaseolus after removing the petals and sepals, we shall observe the monomerous ovary, surrounded by the split filament tube. In Phaseolus and many other Papilionaceæ only nine of the ten stamens are connate, one stamen is free. The ovary bears the ovules on its ventral suture. According to Sachs,[1] the following points in particular are to be observed in the ripening of the fruits and seeds of Phaseolus (P. vulgaris).

Immediately after shedding the petals, no starch is to be found

either in the cells of the outer green layer of the ovary or of the inner colourless layer. Only in the immediate neighbourhood of the vascular bundles in the ventral and dorsal sutures is a small quantity of starch present. The embryo sac contains no starch (perhaps owing to its being very rapidly used up). Starch is, however, present in the neighbourhood of the embryo sac and in the parenchyma of the funicle. When the fruit has attained a length of 3 cm., the outer green layer of the ovary contains starch; the inner colourless layer contains no starch, but much sugar. In the funicle, and in the immediate neighbourhood of the embryo sac, starch is present. The embryo, which is still very small, is devoid of starch. As the development of the fruit and seed proceeds, much starch (but no sugar) is still always to be found in the parenchyma of the funicle, which serves as an organ of conduction, and also a large quantity of starch gradually accumulates in the cotyledons of the developing embryo.

Cases have come before me in which neither in the embryo sac, nor in the tissue of the nucellus and funicle (presumably in consequence of the very active growth of the cells), were starch or sugar to be detected. I investigated on May 4th flowers of Tulipa sylvestris, and found in the cells of the anatropous ovules, contained in large numbers in the trilocular ovary, large quantities of proteid, but neither starch nor glucose.

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[1] See Sachs, Pringsheim's *Jahrbücher*, Bd. 3, p. 231.

128. Preparation of the Material Necessary for Quantitative Chemical Researches on Metabolism.

One of the most important but at the same time most difficult and tedious tasks in quantitative chemical investigations concerning plant metabolism is to obtain suitable material. It is best to use seedlings in such observations, *e.g.* in studying the behaviour of starch or fat. We first endeavour to procure seeds of uniform development, and also thoroughly capable of germination, determine the weight of dry substance they contain, obtaining an average value by drying at 102° C. several samples of the powder obtained by grinding the seeds in a hand-mill, and calculate all the results of the researches in terms of dry seed substance.

The seeds to be used for obtaining seedlings must be accurately weighed; the weight of dry substance they contain is then easily

calculated. The soaking of the seeds, the culture of seedlings, and the determination of the weight of dry substance in these are made as described in connection with the experiments concerning the value of free atmospheric Nitrogen for plants (see 19). The seedlings must naturally, however, be developed in complete absence of light, *e.g.* in a cupboard, and need not be placed under bell-glasses for development.

The chief difficulty in the researches is to obtain uniform seedling material. If we lay out a number of seeds to germinate, it very often happens that many of the seedlings develop vigorously, others feebly, while some may not germinate at all, and decay. In many cases, however (*e.g.* with wheat or peas), we shall be sure to find in a number of cultures some which are thoroughly satisfactory, in which, viz., all the seeds employed have yielded seedlings approximately uniform in development, and that is, of course, the most favourable case to be expected. If we use small seeds (rape, poppy), which, after being soaked on pieces of pumice stone, lying in water, it is best to germinate on moist filter paper or moistened glass wool, it is advisable to count the seeds, and calculate the average weight per seed. The seeds which do not germinate are counted, and we deduct their original weight from the total weight of seeds taken. If the number of seeds germinating badly, or not at all, is not too great, this method does not lead to excessive errors. In researches with large seeds (varieties of beans), it is preferable to deal with each separate object by itself. Each single seed is separately weighed, soaked and germinated on moistened glass wool, and then the development of each separate seedling is continued in a separate glass, containing distilled water, in the manner described for water cultures (see 1). Many little precautions, which are nevertheless of importance to ensure our obtaining seedlings suitable for our purpose, will readily occur to us in carrying out the experiments.

In comparative work on the relation between the metabolic processes and conditions of temperature, the seeds must naturally be germinated in thermostats (see 77) kept at different temperatures.

129. Quantitative Chemical Researches on the Behaviour of Fats and Carbohydrates in Plant Metabolism.*

The study of the processes exhibited in the germination of seeds is very well adapted to explain the behaviour of fats and carbohydrates in metabolism. We first endeavour to determine the percentage composition of the seeds, and of the products of their germination which have been obtained in the manner given in 128, and then, in order to obtain comparable numbers, reduce the results to 100 gr. of seed dry-substance, and the quantity of seedling dry-substance obtained from 100 gr. of seed dry-substance. If, *e.g.*, 100 gr. of seed substance has yielded 90 gr. of seedling substance, the values for the percentage composition of the seedlings must be reduced to a total of 90 gr. The analysis of the seeds and seedlings is made as follows:—

About 3 gr. of the dry substance (seed or seedling) very finely powdered, is extracted with ether in the manner indicated in 120 for determination of the fat. The residue from the fat determination is repeatedly digested for some time with water at the ordinary room temperature. After filtering, the fluid is made up to 200 c.c. Of this, 50 c.c. are used for the determination of sugar (see 115), and 50 c.c. more, after the fluid has been boiled with a little Sulphuric acid, for the determination of dextrin (see 116).

We treat the residue extracted with water, or better about 3 gr. of fresh seed or seedling material (in very fatty seeds or seedlings the fat must first be removed), in a flask of about 500 c.c. capacity, with 200 c.c. of water, boil for some time till the starch has quite thickened, and digest the fluid for about two hours longer at a temperature of 70° C. after adding a few drops of Hydrochloric acid. We then allow to cool, make up to 500 c.c., let settle, and filter off 200 c.c. through a dry filter. We mix the clear filtrate with 15 c.c. of 25 per cent. Hydrochloric acid, boil for three hours, replacing the water lost by evaporation, and after allowing to cool again make up to 200 c.c. In 50 c.c. of the fluid

* Many valuable methods have lately been discovered for accurately determining the quantity of the different bodies present in seeds and seedlings. This is not the place to go into such details. We must hence consult the recent literature on agricultural chemistry and König's book already frequently cited.

we determine the sugar by means of Fehling's solution, and then calculate the corresponding quantity of starch (see 111).[1]

The residue left after treating with Hydrochloric acid, we boil for half an hour with 200 c.c. of 1 per cent. potash solution, filter, and boil the mass remaining on the filter for half an hour with 200 c.c. of water. The residue still left is collected on a weighed filter paper, washed with alcohol and ether, dried and weighed. From the quantity of raw fibre obtained must be deducted the weight of ash and the quantity of proteid present in it, both of which must be determined.

Special portions of the seed and seedling material are taken for the determination of the ash. The quantity of proteid present in the seeds and seedlings must likewise be determined, and possibly also the quantity of asparagin, etc. (see 99).

In calculating the percentage composition of seeds and seedlings from the results of the experiments we have made, there always remains a not inconsiderable deficiency. The quantity of "undetermined substances" must, however, be specified.

As to the significance of quantitative chemical experiments in the study of metabolism, I have spoken fully elsewhere.[2] It need only be mentioned here that by this method we can, *e.g.*, clearly make out in what relation the amount of starch vanishing in metabolism stands to the amount of dry substance disappearing, how much sugar is produced under particular conditions, how much starch is formed in the germination of fatty seeds when a certain amount of fat has disappeared, and so forth. All these questions are of high scientific interest.[3]

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[1] See also König, *Untersuchung landwirthschl. wichtiger Stoffe*, 1891, p. 231.

[2] See Detmer, *Vergleichende Physiologie d. Keimungsprocesses der Samen*, 1890.

[3] See also in the places indicated the literature. Also see Detmer, *Physiol. Untersuchungen über die Keimung ölhaltiger Samen und die Vegetation von Zea Mays*, 1875; Detmer, in Wollny's *Forschungen auf dem Gebiete der Agricultur-physik*, Bd. 2. And Sachsse, *Ueber einige chem. Vorgänge bei der Keimung von Pisum sativum*, 1872.

IV. THE BYE-PRODUCTS OF VEGETABLE METABOLISM.

130. The Organic Acids in Plants.

The organic acids in plants are not to be regarded as products of assimilation. They originate for the most part, as will be more specially shown in 131, from carbohydrates by processes of oxidation. The organic acids of plants (Oxalic, Citric, Malic, etc.) are present in the cell-sap either in the free state or, as perhaps more generally, combined with bases to form acid or neutral salts, which may be readily soluble or soluble only with difficulty. The cell-sap in parenchyma very generally contains more or less considerable quantities of free organic acids or of acid salts of organic acids, and we can readily satisfy ourselves of this by applying blue litmus paper to the fresh-cut surface of any plant structure. The reddening of the paper indicates the presence of the acid. In many cases the acid character of the cell-sap may be detected even by the sense of taste.

The free acids and their acid salts have numerous functions in the cells, which we have already in part considered. The acids very considerably intensify the turgescence of the cell contents, they accelerate the transformation of starch by diastase, they serve in many cases to protect plants against the attack of injurious animals, they decompose the nitrates absorbed from the soil by the roots, a process of great importance in the formation of proteids, they combine with the excess of lime absorbed by the plants, and they decompose the chlorides present in the plant with liberation of Hydrochloric acid.

Oxalic acid is very widely distributed in the vegetable kingdom. It is found free, and in acid salts which are soluble in the cell-sap, but also very frequently in combination with lime. Crystals of Calcium oxalate are found in special cells, and we have referred to them (see 24). Further examples may now be mentioned.

We prepare a longitudinal section, perpendicular to the surface, from the leaf of Aloe arborescens. The epidermis, the green parenchyma, and the aqueous tissue free from chlorophyll are easily made out under the microscope. In the green tissue we notice further tubular cells, running parallel to the long axis of the leaf, which are crowded with large quantities of needle-shaped crystals of Calcium oxalate. These bundles of raphides lie in a mucilaginous matrix, and in the preparation of sections it fre-

quently happens, when any of the tubular cells are accidentally opened, that these mucilaginous masses with the raphides escape, so that we observe them outside the section in the surrounding fluid. If we treat the sections with potash or Acetic acid, the raphides do not dissolve. We further prepare transverse sections from the leaf of Beta vulgaris. On microscopic examination we see clearly the epidermis of the upper and under sides of the leaf, the slightly developed palisade parenchyma, and the spongy parenchyma, with its numerous intercellular spaces. In this last occur the so-called granule tubes, cells which are filled with small crystals of Calcium oxalate. We prepare still further a section from a shoot about 5 mm. in thickness of Tilia parvifolia. The appearance of the transverse section has already been described in 42. We need only notice here that in the outer part of the medullary rays, and in the tissue of the primary cortex, many cells are present which contain cluster crystals of Calcium oxalate.

Some plants, especially the Crassulaceæ (*e.g.* Sempervivum, Echeveria, Bryophyllum) are distinguished by the fact that their cell-sap contains very large quantities of Malic acid, which, as Kraus[1] proved, is for the most part combined with lime. The malate, which is soluble in the cell-sap, sometimes constitutes 50 per cent. of the dry weight of the sap in the leaves of these plants. We pound a few leaves of Bryophyllum in a mortar, transfer the pulp to a dry filter, and determine the dry weight of a small part of the sap obtained, the larger part of it being mixed with 4 or 5 times its volume of 96 per cent. alcohol. The malate separates out in the form of a white powdery precipitate, which we can filter off, wash, dry, and weigh.

It is very often important in plant physiology to determine the acidity of plant saps, *i.e.* the amount of titratable acids which they contain. According to circumstances, different methods must be employed, and we will now consider these. The method of preparing the saps or extracts in which the quantity of acid is to be determined is a matter of prime importance. If in comparative work it is only desired to determine relative values for the acidity, the material when very juicy, as is the case, *e.g.*, with rhubarb leaf-stalks, is rubbed on the grater, or if poor in water is cut up into pieces and pounded as thoroughly as possible in a porcelain mortar. The pulpy masses are squeezed in a cloth by the hand or by means of a press, with as uniform a pressure as possible, and then the sap is finally cleared by filtering. Under

some conditions it is also advisable to lixiviate the crushed masses with a little water, and filter the fluid obtained. If it is desired to obtain absolute values for the acidity of the plant structures, they are first weighed, then the pulp obtained by pounding them is mixed with water and warmed for half an hour in thick-walled glass vessels, in the water-bath, at a temperature of from 80° C. to at most 90° C., to drive off Carbon dioxide, then transferred to a filter and washed with as little hot water as possible (see 60). In investigating the behaviour of free organic acids in the Crassulaceæ (see 131), the best method of procedure is to weigh the leaves which we select for examination, crush them in a mortar, then for half an hour heat the pulp, together with the water employed for rinsing (which should be as little as possible), on a water-bath in thick-walled glasses to a temperature of from 80° C. to at most 90° C., then again transfer to a mortar, rinsing out the vessels with water and pouring the rinse-water into the mortar, and finally after cooling at once titrate.

For titrating saps, extracts or pulp, we use dilute potash or soda lye. We dissolve 1 gr. of caustic potash or caustic soda in 1,000 c.c. of water, add slight excess of baryta water, and then Sodium sulphate in order to precipitate the excess of baryta. The clear solution, which is now free from Carbonic acid, should give no precipitate with Sulphuric acid. To fill the burette we employ an arrangement such as that represented in Fig. 98. In titrating we run the potash or soda solution from the burette into the acid-containing sap, extracts, or pulp, and if we are working with very clear saps, add from 3 to 5 drops of a dilute alcoholic solution of phenolphthalein to serve as an indicator. In other cases, especially in the titration of pulp, turmeric paper must be used. In comparative work it is not necessary to determine the titration equivalent of the soda or potash solution. If, however, this is required, we prepare a normal solution of an acid, *i.e.* a solution which contains 1 equivalent weight in grams of a monobasic acid to every 1,000 c.c. In using Oxalic acid ($C_2H_2O_4 + 2H_2O = 126$) 63 gr. of pure acid must be dissolved in 1,000 c.c. of water. From this solution the equivalent titre of the soda or potash solution is easily determined.[2]

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[1] See G. Kraus, *Abhandlungen der Naturforschenden Gesellschaft zu Halle*, Bd. 16.

[2] On methods of titrating see Mohr, *Lehrbuch d. analytisch-chem. Titrirmethode.*

131. The Behaviour of the Free Organic Acids in the Crassulaceæ, and Some Other Plants.

Many Crassulaceæ and other plants, especially succulent ones, are very remarkable in the fact that the quantity of free organic acids in their sap (we have in these cases specially to deal with Malic acid) is much less in the daytime than at night. The extremely complicated relations in question have been by no means thoroughly examined in all directions. But a few facts have been established, and shall be experimentally confirmed below, after I have first stated very briefly the views which I have formed on the basis of previous investigations as to the behaviour of organic acids in the Crassulaceæ.[1] In the tissue (especially in the leaf tissue) of the Crassulaceæ and some other plants, two processes are constantly and under all circumstances going on side by side, which appear to be of the utmost significance in connection with the acidity of the sap. On the one hand, production of acid is always taking place, and on the other, acid is always undergoing decomposition. The amount of acid at any given moment is therefore the resultant of these two processes.*

It is a fact of great importance that, under certain conditions, considerable quantities of free acid can accumulate very rapidly in the cells of the Crassulaceæ, and one which is undoubtedly of biological significance for the plants. These, viz., grow generally in dry and often very calcareous localities. They need therefore special means for increasing the osmotic capacity of their cell contents, so that large quantities of water may collect in their tissues, and at the same time for rendering possible combination of the excess of lime transferred to their cells from the soil. The organic acids serve both these ends.

The acids are produced from carbohydrates under the influence of Oxygen. They originate from oxidation of the products of assimilation. That such considerable quantities of acid should accumulate in the tissue of succulent plants is related to their organization. Succulent plants, viz., in virtue of the possession of a thick cuticle, relatively few stomata, and fleshy tissue, maintain

* Besides the decomposition of free organic acids in the tissue of the Crassulaceæ there is continually going on in them a combination of the acids with bases (Kraus). Hence large quantities of certain salts gradually collect in the cells, and the production of these compounds, which however we must here leave out of consideration, is also naturally not without significance as regards the acidity of Crassulaceous plants.

only a very limited interchange of gases with the exterior. Oxygen is not at the disposal of their cells in excessive quantity, and the combustion of the carbohydrates is therefore only incomplete. It does not, under certain conditions at least, proceed as far as the production of Carbon dioxide and water, but considerable quantities of organic acids accumulate as products of incomplete oxidation.

The process of acid decomposition which is constantly going on side by side with the process of acid formation in the tissue of the Crassulaceæ is, as regards the energy with which it proceeds, highly dependent on conditions of temperature and illumination. High temperature and exposure to light very considerably accelerate the decomposition of the acids. Hence the acidity of the tissue in Crassulaceous plants diminishes when they are exposed in the dark to a high temperature, or when they are submitted to the influence of light. In darkness, on the other hand—especially at a low temperature—the acidity of the sap of the Crassulaceæ rises. And indeed we know that the acidity of the sap in these plants undergoes a daily periodic variation. In the daytime the sap is slightly acid in reaction, at night strongly acid.

The decomposition of the acids is essentially an oxidation associated with production of Carbon dioxide. The acid already formed by oxidation undergoes further and complete oxidation, and the question now arises how it happens that the rays of light accelerate the process. It has been already mentioned that the Crassulaceæ can for various reasons carry on only a limited interchange of gases. This determines the collection in the tissue of large quantities of acid. In darkness there is a deficiency of Oxygen in the tissues; access of light, on the other hand, markedly increases the quantity of Oxygen in the tissues, since Oxygen is set free in assimilation. The assimilating chlorophyll grains do not take part directly in the process of acid decomposition, but they do indirectly by causing the liberation of considerable quantities of Oxygen in the tissues, which in turn effect complete oxidation of the acid. The Carbon dioxide thereby originating can again be worked up in the chlorophyll corpuscles, and the Oxygen set free is once more in a position to accelerate the decomposition of acid.

These relations are of the very first significance in explaining the increased decomposition of acid, and they are of themselves sufficient to account for the acceleration of this process under the influence of light. But it appears probable to me that the rays of

light also participate directly in the action. We shall make experiments which go to show that access of light favours the oxidation of organic acids outside the organism. A direct influence of the rays of light on the process of acid decomposition in the cells of plants therefore appears to be quite possible.

We know that if parts of succulent plants are left for some time (*e.g.* for a night) in a limited quantity of air, they cause a diminution in its volume. Oxygen, in fact, enters into combination with formation of organic acids. The same structures, on the other hand, increase the volume of the air surrounding them in the daytime, since they expire Oxygen. This Oxygen is, however, not directly split off from the molecules of the decomposing organic acids, but is a product of the actual assimilatory activity of the chlorophyll corpuscles. The necessary Carbon dioxide is, however, afforded in the manner above discussed by the decomposing organic acids.

We now proceed to give directions for experiments which will give information concerning the behaviour of organic acids in the Crassulaceæ.

For our observations we employ vigorous pot-plants, grown under the most favourable external conditions, of Bryophyllum calycinum, Echeveria metallica, or Rochea falcata (the last plant is particularly suitable). In comparative work on the accumulation or disappearance of acids in the tissue of the leaves, we employ either the two opposite leaves of a pair (*e.g.* Rochea), or we experiment with a single leaf only (*e.g.* Echeveria metallica), and divide it longitudinally into two portions as nearly as possible similar. The objects are weighed immediately after removal from the plant. If it is not required to determine the quantity of free acid which they contain until some time has elapsed, they are placed, *e.g.*, on moistened blotting-paper, and covered with bell-glasses. We determine the amount of acid in the leaves by titration. The quantity of potash used in titrating gives a direct measure of the acidity of the cell-sap (for method see 130).

We first carry out the following investigation :—A plant of Rochea is exposed during the day to direct sunlight, and towards evening, about five or six o'clock, a pair of leaves are cut off. If we experiment with Bryophyllum, we take a few pairs of leaves ; in experiments with Echeveria we use only one of the large leaves. Half of the material is at once tested acidimetrically, the other half next morning, the objects having been kept till that time in

complete darkness in moist air under a bell-glass. I found, *e.g.*, that the two members of a pair of Rochea falcata leaves weighed respectively 12·6 gr. (*a*), and 13·6 gr. (*b*). *a* was examined in the evening, immediately after being cut off, *b* not till the next morning. The pulp from *a* required for neutralisation 2·6 c.c., that from *b* 12·5 c.c., of dilute potash. For every 10 gr. of leaf substance employed these numbers give for *a* 2·1 c.c., for *b* 9·2 c.c., of potash, a difference of 7·1 c.c. Another plan is to cut only one leaf from the plant in the evening, and determine its acidity at once, while the second leaf is not removed till the next morning, the plant having meanwhile been kept in complete darkness. The experiments always indicate a considerably higher acidity in the case of leaves which have been kept some time in darkness.

To prove still more certainly that the acidity of the sap of Crassulaceous plants diminishes in presence of light, but increases in darkness, it is necessary to make the following experiment:—Two opposite leaves of a plant of Rochea are cut off early in the morning. One leaf is halved longitudinally, and in one half, after weighing it, we at once determine the quantity of acid in the cell-sap by titration. The second half we hang under a bell-glass, the air within which is saturated with moisture, and exclude the light by covering over with a cardboard cylinder. The second leaf is hung up under a bell-glass, the space within which is also saturated with moisture, and exposed to very bright diffuse daylight, care being taken that the back of the leaf also receives light reflected from a suitably arranged mirror. Proceeding in this way the objects are exposed to approximately the same conditions of temperature, and diminution of acidity due to rise of temperature is, as far as possible, excluded. If in the evening we determine the acidity of the half leaf and of the entire leaf, we shall find that the former is richer in acid than the latter (comparing naturally equal weights of fresh leaf substance). We easily satisfy ourselves of the important influence of temperature on the process of acid decomposition in the leaf of Crassulaceous plants, if in the early morning we remove a few of the acid leaves from plants of Bryophyllum, Rochea, or Echeveria, which have been exposed to normal conditions of environment, and in some of them determine the acidity at once, while in the remainder the acidity is determined after they have been kept in complete darkness for about twelve hours, part at a lower temperature (say 12°–16° C.), part in a thermostat at a temperature of 30° C. It will appear that

the exposure to the higher temperature, in spite of the exclusion of light, has brought about a diminution in the acidity.

Towards evening we take a pair of leaves from a plant of Rochea (we may also experiment with Echeveria or Bryophyllum). In one leaf the acidity is determined at once, the other is longitudinally halved. Each half, after being weighed, is cut up into small pieces about 1 cm. long, and these are placed in retorts filled with distilled water which has been boiled and then allowed to thoroughly cool again. The water in one retort is replaced by air, that in the other by pure Hydrogen (for method see 10). Next morning we make determinations of acidity, and it is found that the pieces exposed to air have produced much free acid, while in those exposed to Hydrogen very small quantities at most of free acid have been formed. Free Oxygen is therefore necessary for copious production of acid.

It has already been mentioned that access of light accelerates the decomposition of organic acids outside the organism, a fact which, as I previously remarked, is most certainly of interest in connection with the question which we are here considering. We can easily demonstrate (even in lecture) the influence of light on the decomposition of acids. We fill a test-tube to the top with a $\frac{N}{5}$ solution of Oxalic acid, add to it some freshly prepared Ferric hydrate (prepared by mixing Ferric chloride solution with ammonia and carefully washing the precipitate), and now invert the test-tube over mercury and expose the solution, which, after some time, becomes intensely yellow in colour, to direct sunlight. An evolution of gas at once begins; the gas (Carbon dioxide) collects in the upper part of the tube, while the fluid becomes colourless, and a precipitate of Ferrous oxalate separates out. It is not impossible that the oxidation of organic acids in the plant is similarly greatly promoted by the direct influence of rays of light.

Finally, we will make experiments which teach that succulent structures, as already mentioned, actually take up a comparatively large quantity of Oxygen, when acids are accumulating in their cells. For the purpose of lecture demonstration it is quite sufficient to proceed as follows:—We take a leaf from a plant of Rochea falcata on the evening of a hot summer day (the leaf which I used weighed 24 gr.), cut it up into pieces, and place the pieces in the upper expanded part of the eudiometer shown

in 10, Fig. 11. We dip the lower end of the eudiometer in water, close the apparatus, and place it in the dark. After some time (*e.g.* twelve hours) we find that the water has risen considerably in the tube, while this is not the case in parallel experiments in which young stems of non-succulent plants (*e.g.* Helianthus) were introduced into a second eudiometer. The pieces of Rochea leaf carry on not only normal respiration, but at the same time vinculatory respiration. They absorb, in fact, much Oxygen, without corresponding production of Carbon dioxide, for the conversion of carbohydrates into organic acids.

In exact quantitative investigations concerning the inspiration of Oxygen by succulent plants we must naturally employ mercury for closing the eudiometer. The method of procedure will be obvious from what was said in 13.

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[1] Literature: A. Mayer, *Landwirthschl. Versuchsstationen*, Bd. 18 and Bd. 21; Detmer, Pringsheim's *Jahrbücher*, Bd. 12, and *Lehrbuch der Pflanzenphysiologie*, 1883; H. de Vries, *Verslagen en Mededeelingen der Koninkl. Akadem. van Wetenschappen*, 1884; G. Kraus, *Abhandlungen der Naturforschenden Gesellschaft zu Halle*, Bd. 16; Warburg, *Untersuchungen a. d. botan. Institut zu Tübingen*, Bd. 2. The views which I have expressed in my cited papers on the subject before us are essentially different from those here advocated by me.

132. Gums and Mucilages.

Gum arabic (the product of various sorts of Acacias) consists chiefly of Arabic acid. If we treat some gum arabic in a watch-glass with iodised Potassium iodide solution, and then add Sulphuric acid, the mass only assumes a brown coloration. All the true gums behave in this manner, while mucilages take on a violet or blue coloration on treatment with Iodine and Sulphuric acid. According to the researches of Von Mohl, gum tragacanth is produced by the disorganisation of the cells of the pith and medullary rays in different kinds of Astragalus. Gum tragacanth is not a homogeneous mass, as we can see at once if we treat the commercial and powdered substance with a large quantity of water. A solution is produced which on evaporation yields a colourless glassy mass, gum tragacanth proper, and a sediment which under the microscope is seen to consist of starch-grains and fragments of cell-walls. The amount of cell-membrane incompletely converted into gum varies in different kinds of tragacanth.

Gum reservoirs are readily detected if we examine sections

from twigs of Tilia parvifolia about 5 mm. thick. The air-containing cells of the pith are large. They are grouped in the form of rosettes round single small cells which contain tannin, starch, or cluster crystals. The gum reservoirs, appearing as cavities, lie in the outer parts of the pith. The periphery of the pith, into which the primary masses of wood project, is formed of small-celled parenchyma whose elements contain tannin or starch.

If we examine under the microscope a transverse section from a tuber of Orchis mascula, or O. Morio, the parenchyma in which the vascular bundles are scattered is seen to consist of small cells which contain starch, and large cells which are very rich in mucilage. If we extract pulverized Orchid tubers with cold water, or treat commercial salep with cold water, we shall obtain after filtration a clear fluid. Addition of alcohol to this causes precipitation of white flocculent masses of Orchid mucilage, insoluble in alcohol. If we evaporate the mucilaginous solution prepared as described, and treat the residue with iodised Potassium iodide solution and Sulphuric acid, it takes on a violet to blue coloration. The mucilaginous masses in Orchid tubers are thus not gum but true vegetable mucilages.[1]

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[1] The literature on gums and mucilages is given in Sachsse, *Die Chemie und Physiologie der Farbstoffe, Kohlehydrate*, etc., Leipsic, 1877, p. 161.

133. Tannic Acids.

Tannic acids appear to function in plants chiefly as a means of protection against the attacks of animals, and as antiseptics. It is quite in accordance with this that in many cases it is precisely the peripheral tissues which are specially rich in Tannic acid. The best test for Tannic acid is Potassium bichromate,[1] and we will make use of it to determine the distribution of Tannic acid in a shoot of Corylus Avellana. We first prepare transverse sections from a twig about 4 mm. thick. The periderm is followed by collenchyma, this by cortical parenchyma; then comes a ring of strongly thickened sclerenchyma cells, followed by the bast with its scattered bast fibres, and lastly the wood. If we put longitudinally halved pieces of Corylus twigs (I investigated twigs 4 mm. thick, cut in November) for a few days into a 10 per cent. solution of Potassium bichromate, and then examine under the microscope delicate transverse sections, the presence of Tannic

acid in certain tissues will be easily made out (particularly in the cortex, bast parenchyma, and the usually unilayered medullary rays). The contents of the cells containing Tannic acid are coloured reddish brown.

On examining longitudinal sections from the pith of a current year's rose shoot, we see that it consists on the one hand of large cells, and on the other of longitudinally running rows of cells communicating with one another, which traverse the large-celled tissue. If we examine sections from the pith, mounted in a drop of a 10 per cent. aqueous solution of Potassium bichromate, we find that the contents of most of the narrow cells are stained reddish-brown. We can also detect the Tannic acid in the narrow cells by placing sections from the pith in a drop of aqueous Ferric chloride solution, or in a drop of Ferric sulphate solution. The contents of the cells containing Tannic acid then stain dark blue. In roses the leaves also are very rich in Tannic acid, and to demonstrate this, *e.g.* in lecture, we fold up a leaf and crush it with the finger on white blotting-paper, so as to squeeze out some of the cell-sap. On touching the moistened parts of the blotting-paper with a solution of Ferric chloride, the Tannic acid reaction at once appears.

Recently several attempts have been made to investigate quantitatively the occurrence of tannins in plants, in order to obtain a clearer understanding of the processes which bring about the production of tannins in the organism. In these investigations, and also in microchemical studies of the genetic relationships of tannins, it must not be forgotten that the substances designated *tannins* may be of very different chemical constitution, and hence all work in this direction must still be of a provisional nature. Such researches nevertheless merit, as closer study of them teaches, careful consideration, and we must not dismiss them without attention.

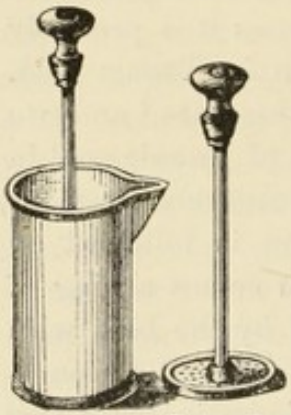

Fig. 114. — Extraction apparatus for tannin determinations.

For quantitative estimations of tannin we employ material dried at 100° C. Bark, woods, or massive rhizomes are ground up very finely in a suitable mill.[2] Delicate rhizomes or roots, leaves, seeds and seedlings are soaked in water and then rubbed down as finely as possible on a grater.[3]

For extraction of the tannin the arrangement represented in Fig. 114 is very suitable. The apparatus, which is made of tin, consists of a lipped cylinder 12·5 cm. in height and 7 cm. in internal diameter, and of a rod carrying at one end a sieve plate. Before using the apparatus, a piece of thin gauze is very carefully tied over the sieve. 2–5 gr. of the research material, dried at 100° C., are treated with 200 c.c. of water, and after twelve hours the fluid is poured into a litre flask. The residue is now transferred to the extraction apparatus, and four times digested at boiling point for half an hour each time, with 200 c.c. of water, the cylinder being placed for the purpose in a water-bath. The extract is finally filtered. The tannin in the filtrate is accurately determined by the Löwenthal–v. Schröder method, as described in the Report cited in Note 3, by titrating with chameleon solution [standard solution of Potassium permanganate] and Sodium sulphindigotate (Carminum cœrul. opt. of Gehe and Co., Dresden). The volume of the tannin-containing solution used in any particular titration must be such that the volume of the chameleon solution needed for its reduction is not much less than that required for the indigo solution (10 c.c.) alone. From the volume of chameleon solution required is determined the percentage of tannin so-called, on the assumption that 1 c.c. of the chameleon solution corresponds with 2 mgr. of tannin.

Among the most important facts determined by Kraus in his cited work are these, that tannins are produced in green leaves in the light and in presence of Carbon dioxide, but not in the dark or in absence of Carbon dioxide. Nevertheless, tannins are not to be regarded as products of assimilation; more probably they originate as bye products of metabolism in the synthesis of proteids. Many plants, moreover, in general do not form tannins, and often even in organs which normally contain them they are not produced, although carbohydrates are being formed by assimilation. This last is the case, *e.g.*, in comparatively feeble light.

We cut in summer, in warm weather, six mature leaves of Saxifraga crassifolia, remove one half of each leaf by section alongside the midrib (without, however, injuring this), and from the isolated halves remove pieces, each about 50 sq. cm. in area, using templets made of millimetre paper. We now dry these pieces of leaf, and determine the quantity of tannin they contain. The half-leaves left attached to their midribs are placed with

their stalks dipping into spring water contained in small glasses, and these are then put into a glass receptacle, which may, if necessary, be covered with a sheet of glass. Thus arranged, we expose the material to the light in the slight shade afforded, *e.g.*, by birch trees. After a few days we treat these halves like those already dealt with, cutting out pieces with the help of our templets, from exactly corresponding places of course, and determining the dry weight of these pieces and the amount of tannin they contain. Leaves of Quercus and Viburnum may be used for similar experiments. We shall find that our leaves exposed to the light gain in dry weight and in tannin; that is not the case if the leaves are kept for a few days in the dark. Making similar experiments with leaves of Saxifraga, with the modification, however, that the leaves are exposed to the light under a large bell-glass in an atmosphere devoid of Carbon dioxide, it is found that no increase in the quantity of tannin takes place. We have therefore made out that tannin can only be produced in green leaves under the influence of light and in presence of Carbon dioxide (indirect influence of assimilation).*

In the evening of a warm summer day we cut off half the leaf as above from ten leaves of Alnus glutinosa, the other half being left attached to the plant. We then determine the tannin in 150 sq. cm. of the separated halves. Next morning the other halves are tested. Loss of tannin has taken place. The searching investigations of Kraus (p. 9 of his cited work) teach that the tannin does not undergo decomposition in the leaves, but migrates out of them. It travels along the leaf-nerves into the stems or branches, proceeds by the cortex, and ultimately is deposited according to the kind of plant in the rhizomes, the wood, or elsewhere. This primary tannin formed in the leaves does not again enter into metabolism. It serves particularly to protect the organs in which it is deposited, as a protection against being eaten by animals, and against decay.

Besides the primary tannins, however, there are the so-called secondary tannins, which in many plants are formed in darkness as a result of metabolic processes. If we test seeds of Vicia Faba we find them to be devoid of tannin. If now we examine seed-

* For the material used and under the conditions obtaining in our experiment, this is certainly correct. Under modified conditions, however, the result may be somewhat different. See Büsgen, *Beobachtungen uber das Verhalten des Gerbstoffes in den Pflanzen*, Jena, 1889, p. 28.

lings of Vicia grown in the dark, they prove to be very rich in tannins.

If it is desired to study the formation and subsequent behaviour of tannins microchemically, it is best to proceed as follows:—We inject the objects under the air-pump with a 10 per cent. solution of Potassium bichromate, let them die in it, and then, after careful washing, examine them, either at once or after preserving in alcohol. By this means we readily determine,[4] *e.g.*, that in seedlings of Vicia Faba grown in darkness, secondary tannin appears in specially large quantities in the epidermis and sub-epidermal tissue, and in the parenchyma surrounding the vascular bundles. The main root of the seedling contains much tannin in the cortex; its central cylinder is almost devoid of tannin.

Secondary tannin is also contained in the stems of seedlings of Phaseolus multiflorus, grown in the dark. It occurs in special tubes in the sieve region of the vascular bundles. The radicle of Phaseolus does not contain tannin; the embryo also of dormant Phaseolus seeds contains no tannin at all. To detect the tannin we employ in this case likewise a solution of Potassium bichromate or of Ferric chloride.

We cut off in spring twigs of Lonicera tatarica, and put them with their base in water. The buds of some of the twigs are allowed to unfold in the dark, those of others in the light. Primary tannin can only be detected by microchemical means in the axis of the young shoots which have developed in the light (*e.g.* in the epidermis and cortical parenchyma). The shoots produced in the dark are devoid of tannins. For further information, see Büsgen's treatise.

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[1] See Sanio, *Botan. Zeitung*, 1863, p. 17.

[2] If the material with which we are dealing is not too hard, we may use one of the so-called Excelsior mills, to be obtained at a price of 30–40 mks. from Gruson in Magdeburg-Buckau. For grinding woods, etc., suitable mills are to be obtained of G. Wenderoth in Cassel.

[3] For further particulars see Kraus, *Grundlinien zu einer Physiologie des Gerbstoffs*, Leipzig, 1889, and *Berichte über die Verhandlungen der Commission zur Feststellung einer einheitlichen Methode der Gerbstoffsbestimmung*, Fischer, Cassel, 1885.

[4] See Büsgen, *Beobachtungen über das Verhalten des Gerbstoffes in den Pflanzen*, Jena, 1889.

134. Ethereal Oils and Resins.

Ethereal oils in many cases are certainly not to be regarded as excreta but as secretions, *i.e.* as bodies with definite physiological functions (to attract creatures necessary for the transference of pollen, to keep away injurious creatures, etc.). Similarly many resins must be considered as secretions.[1]

Ethereal oils are frequently present in intercellular spaces. If we examine, *e.g.*, transverse sections from the stem of Ruta graveolens, taking care that they are not too thin, we find under the epidermis a hypoderm followed by green parenchyma. In this last occur here and there spaces filled with a yellowish highly refringent fluid (ethereal oil). Intercellular secretory spaces filled with ethereal oil are likewise readily made out in transverse section of the leaf of Citrus. The ethereal oils dissolve in alcohol.

Ethereal oils, however, do not only occur in intercellular spaces, but also within cells. Secretory reservoirs of this kind, and especially those relegated by de Bary to the category of "short tubes," because the cells in question are nearly isodiametric, are found in Aristolochia Sipho. If we examine transverse sections from stems about 4 mm. thick, we see at once the pith and the vascular bundles with their wood and bast. Each vascular bundle is bounded on the outside by cortical parenchyma, this being followed by a closed ring of sclerenchyma, which projects inwards somewhat between the vascular bundles. Outside this ring of sclerenchyma comes parenchyma, then collenchyma, and finally the epidermis. In the cortical parenchyma lying outside and inside the ring of sclerenchyma, we now observe, both in transverse and longitudinal sections, scattered cells with yellowish highly refringent contents. These are the secretory reservoirs with which we are concerned. Toward alkanna tincture and Osmic acid the ethereal oils behave like fats. If, however, without putting on a cover-glass, we heat the sections on the slide for ten minutes in the drying chamber at a temperature of 130°, the ethereal oils disappear, since they are volatile.

It is further instructive to verify the fact that the fruits of many Umbelliferæ are rich in ethereal oil, occurring here in intercellular spaces, and evidently functioning as a protection against injurious animals. We prepare, *e.g.*, transverse sections through the laterally compressed fruit of Carum Carvi. Each

half of the fruit is filled with endosperm in the midst of which is the embryo. We further see the five main ribs of each mericarp. In the tissue of the wall of the fruit we perceive the vittæ, which are intercellular spaces, filled with ethereal oil.

To observe resin passages we may examine very delicate transverse sections of the needles of Pinus sylvestris. The epidermis, whose cells are very strongly thickened, is followed by a layer of hypoderm cells. At the two angles of the leaf this layer is more strongly developed. The resin passages (quite a number of them are always present) lie in the green tissue. Each resin passage is lined by a layer of thin-walled cells, the epithelium, which without doubt yield the material from which the secretion is formed, and furthermore, each resin passage is surrounded by a layer of strongly thickened sclerenchyma fibres. We see also the green tissue of the leaf, and the tissue, almost devoid of chlorophyll, in the middle of the leaf, which is separated from the green tissue by an endodermis. The nearly colourless ground tissue of the middle of the leaf is seen to be made up of thick-walled and thin-walled elements, and encloses two vascular bundles. The leaf of Pinus Pinaster is similar in construction to that of P. sylvestris. Its leaves are better for section-cutting than those of P. sylvestris, and if they are available we give them the preference for this reason. It is also very easy to make out the presence of resin passages in the stems of many Umbelliferæ. We examine, *e.g.*, under a low power, transverse sections from the flower stalk of Fœniculum officinale. The epidermis, the cortex, the bast and wood of the vascular bundles, and the pith are clearly seen. The resin passages are situated in front of the vascular bundles. They lie in the cortex between the fibro-vascular strands and a tissue which we at once recognise as collenchyma.

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[1] See de Vries, *Landwirthschl. Jahrbücher*, Bd. 10.

135. Colouring Matters.

Many plant structures contain pigments of very different kinds. In the first place it must be emphasised that these pigments are of no slight physiological interest, from the mere fact that many of them indicate directly the reaction of the cells in which they are found. In the cell-sap of the hairs on the leaf-stalk of many Begonias are dissolved red pigments, whence we conclude that the

reaction of the cell-sap is acid. If a hair is treated on a slide with very dilute potash solution, the colour of the pigment is in fact changed to blue; on the addition of acid the red colour again appears. In the cell-sap of the cells of Myosotis petals a blue pigment is dissolved. The reaction of the sap is in this case faintly alkaline. Addition of acid causes a change to red.

If we examine under the microscope the hairs of the filaments of Tradescantia stamens, we can readily make out that a violet pigment is dissolved in the cell-sap of their cells. With the forceps we tear a strip of epidermis from the petal of a Vinca and of a red rose. In both cases the cells are seen under the microscope to contain dissolved pigments. In one case, however, the pigment is blue, in the other rose-coloured. The blue, violet, or red pigments dissolved in the cell-sap are classed as anthocyan.

Many pigments occur in the cells not in solution, but associated with a matrix. The colour corpuscles (chromatophores) impregnated with pigment are for the most part of characteristic form, and we first select for examination not too ripe but still well-coloured hips. We prepare sections from the flesh of the hypanthium. The cells contain, besides protoplasm and nucleus, pointed orange-coloured spindles or similarly coloured triangular bodies which are the chromatophores. The orange-red colour of the roots of carrot plants (Daucus carota) is due to the presence in the cells of chromatophores, which are easily made out under the microscope as rectangular plates or elongated prisms. We further prepare tangential sections from the upper side of the sepals of a just opened flower of Tropæolum majus. In the cells, especially the epidermal cells, are revealed, on microscopic examination, many angular chromatophores, yellow in colour (see Fig. 115). The brown streaks on the upper side of the sepals of Tropæolum are due to the fact that the corresponding cells of the epidermis contain a carmine-coloured cell-sap, as is readily proved by study of suitable sections. The yellow pigments of plants are almost without exception associated with a protoplasmic matrix. Only rarely do we meet with them dissolved in the cell-sap. This is the case, however, in the epidermal cells of the petals of Verbascum nigrum.[1] The pigment of most yellow flowers is not soluble in water, but dissolves in alcohol. By means of this solvent, *e.g.*, it can readily be extracted from the petals of a yellow-flowered Ranunculus. The pigments of most red flowers on the other hand are soluble in water, and if the petals, *e.g.*, of

a red rose or peony are macerated with a little water in a mortar, the solution obtained on filtering is red in colour, and according to my observations (I experimented with Pæonia) becomes blue on addition of ammonia. Addition of Hydrochloric acid restores the red colour of the fluid. It may also be of interest to examine the extracts from yellow or red flowers spectroscopically.[2] For this purpose the methods given in 7 are to be employed.

It is also instructive to observe the pigments contained in the heart-wood of many trees. We examine, for example, a transverse section of red sandal wood (the wood of Pterocarpus santalinus).

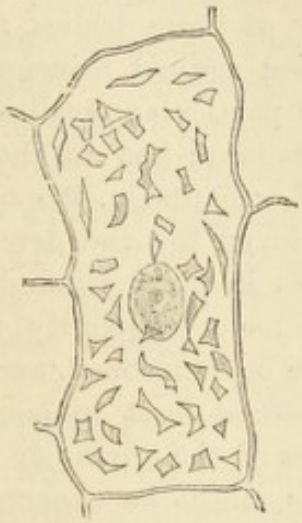

FIG. 115.—From the upper side of the calyx of Tropæolum majus. Lower wall of an epidermal cell with the adjacent chromatophores. Magn. 540. (After Strasburger.)

The wide vessels rest against the bands of wood parenchyma, which run parallel with the annual rings. We further see the numerous medullary rays, whose cells contain a resinous dark red mass, and the wood fibres with strongly thickened walls. All the elements of the wood have pigments in their membranes consisting specially of the red Santalic acid. Distilled water only extracts traces of pigment from sandal wood, but with ammonia we easily obtain a carmine red extract.

The elements of Brazil wood (Cæsalpinia echinata) hold in their membranes a yellowish pigment, brasilin. If we treat Brazil wood with hot water, a fair amount of this pigment goes into solution, and the fluid takes on a blood-red coloration on addition of ammonia or potash.[3]

[1] On some of the points here referred to see Strasburger's *Practical Botany*.

[2] See Hansen, *Verhandlungen der Physicalisch-medicinischen Gesellschaft zu Würzburg*, Neue Folge, Bd. 18, No. 7.

[3] Respecting the anatomical structure of Brazil wood, see Wiesner, *Rohstoffe des Pflanzenreiches*, 1873, p. 555.

136. Micro-chemical Tests for Alkaloids and Some Other Substances.

It is well known that in the tissues of the most different plants there are present alkaloids, glucosides, or other substances, regarding the physiological significance of which little is up to the present known. Many of these bodies, there is no doubt, serve as a means of protection against injurious animals; others also (*e.g.* glucosides), under particular conditions, may decompose and yield plastic material (sugar); but all these relations have as yet been but little studied. Similarly, the micro-chemical reactions which have been employed for the detection of at least some alkaloids and glucosides in plant tissues, are still in some cases rather uncertain, as I have frequently found. Nevertheless, a few reactions may here be mentioned, and we shall find that the substances in question are at least frequently collected especially in the peripheral tissues of the organs or in the neighbourhood of the vascular bundles, as might be expected if they function as a means of protection against animals.[1]

If we examine a thin section from the horny endosperm of the seed of Strychnos nux vomica, the cells are at once seen to be rather thick-walled. Their contents are proteids, sugar, and fatty oil. If thin sections from the dry seeds are mounted in a drop of concentrated Sulphuric acid, the cell contents in the course of a few minutes take on a reddish colour. We now add to the section lying in Sulphuric acid a fragment of Potassium chromate, cover with a cover-glass, and observe under the microscope. The cell contents, especially those of the endosperm cells immediately below the testa, rapidly take on a beautiful violet coloration, while the cell-walls remain uncoloured (strychnine reaction).[2]

In transverse sections from the stem or branches of Berberis vulgaris (we may, *e.g.*, use pieces from twigs about 6 mm. thick) we easily distinguish the cortex[3] and the vascular bundles. In the cortex, the soft bast, and the phloem rays, appear many cells with yellow contents, the colour being due to the presence of

berberin. Berberin is also found in the peripheral part of the wood (viz., as a deposit in the membranes).

On treating the sections with alcohol and very dilute Nitric acid (1 part of Nitric acid to 50 parts of water), the yellow colour disappears from the berberin-containing elements. The presence, however, of large quantities of berberin now causes the separation of yellow crystals of Berberic nitrate.

We prepare delicate transverse or longitudinal sections from a corm of Colchicum autumnale. In the immediate neighbourhood of the vascular bundles we observe cells which contain a strongly refringent yellowish fluid, while the main mass of the parenchyma is very rich in starch. These yellow cells contain colchicin. On treating the sections with ammonia, their contents take on an intensely yellow coloration. The roots of Colchicum contain colchicin in the epidermis and the protective sheath.

In the stem of Aconitum Napellus, aconitin is found in the vascular bundle sheath and in the parenchyma in its neighbourhood. Aconitin gives with iodised Potassium iodide solution a brownish-red precipitate; and with Sulphuric acid diluted with $\frac{1}{2}$–$\frac{1}{3}$ its volume of water, especially if the preparations have previously been treated with cane-sugar solution, it gives a carmine red coloration (Errera).

If transverse sections from twigs, about 3 mm. thick, of Syringa vulgaris are mounted in dilute Sulphuric acid (1 part by volume of concentrated Sulphuric acid and 2 parts by volume of water), the membranes of the wood elements, of the medullary rays in the wood, and of the bast fibres colour yellowish green, and later bluish green. All the other cells remain uncoloured. The reaction in question is caused by the presence of syringin. This substance is deposited in the membranes. Sometimes, also, the contents of the cells of the cortex take on a bluish colour when sections of Syringa are treated with Sulphuric acid; but this is merely due to some of the syringin having diffused into these cells in the course of the reaction.

We prepare transverse sections from twigs of Rhamnus Frangula, about 3 mm. in diameter, and treat them on the slide with an alcoholic solution of Potassium hydrate. Various elements of the sections, especially the thin-walled elements of the bast, become intensely red in colour, the coloration being, however, not very stable (frangulin reaction).

In transverse sections from the root of Rumex crispus, we shall

easily distinguish the cork, the cortex, and the bast and wood of the vascular bundles. In the somewhat older parts of the root the wood forms a closed cylinder traversed by medullary rays. If the sections are treated with dilute potash solution, the contents of the thin-walled elements of the cortex and phloem take on an intensely red colour, which is very stable (Chrysophanic acid reaction.)[4]

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[1] See Errera, *Botan. Centralblatt*, Bd. 32, p. 71.

[2] Rosoll, *Sitzungsber. d. Akadem. d. Wiss. zu Wien*, Abthl. 1, Bd. 89.

[3] An exact account of the structure of the Berberis stem, and especially of its cortex, will be found in a Königsberg *Dissertation* by Böning, of the year 1885, *Ueber die Anatomie des Stammes der Berberitze.*

[4] See Borskow, *Botan. Zeitung*, 1874, and O. Herrmann, *Leipziger Dissertation*, 1876.

137. The Bye Products of Plant Metabolism as Means of Protection to Plants.

Many creatures, particularly mammals, insects, and snails, would be exceedingly prejudicial to the life of plants, if these were not more or less protected. The means of protection are partly mechanical, partly chemical, and we often find, though certainly not always, that plants which are protected mechanically, do not produce any so-called chemical means of protection, and *vice versa.*

Many bye products of plant metabolism, as has already been pointed out several times, have especially the object, among other things, of providing the plant with a chemical protection against animals, and we will here, with Stahl's researches[1] as a basis, undertake a few experiments by means of which this fact can easily be proved. Our object is to show that tannins, vegetable acids, ethereal oils, etc., afford the plant protection from snails.

We collect a number of snails, *e.g.* Helix pomatia and H. hortensis. For two to three days the snails are not supplied with food, so that when the experiments proper begin they may be very hungry. They are now placed in good-sized crystallising glasses, two or three in each if Helix pomatia is used, larger numbers if smaller kinds of snails are used, and the glasses are then covered with glass plates, which are weighted, so as to prevent the snails from creeping out. The snails are now supplied with various kinds of foods. We will experiment with the

following kinds of leaves: Trifolium pratense (tannin-containing), Rumex, Oxalis (acid), Ruta graveolens (rich in ethereal oil), Ranunculus acris, and Tropæolum majus. In each crystallising glass are placed with the snails one or two fresh leaves, but at the same time we supply leaves from which the chemical protective material has been extracted by warming with alcohol, and then drying in the sun or drying chamber, and finally washing with distilled water. Since the snails feed chiefly in the evening and at night, it is always advisable to put the glasses with their contents aside for a good time. The fresh leaves are not at all or only little attacked by the snails; they rapidly destroy the extracted leaves. The following experiment also definitely teaches that, *e.g.*, tannins and vegetable acids must afford plants an excellent means of protection against snails. We supply the snails in the crystallising glasses with thin slices of carrot (Daucus carota), and at the same time with slices which have been killed by boiling water, dried in the oven, and finally soaked in a 1 per cent. solution of tannin, or a 1 per cent. solution of acid Potassium oxalate. The fresh pieces of carrot are very readily eaten by the snails; the slices impregnated with tannin or acid they reject entirely.

We must not fail to make the following experiments in order to acquaint ourselves with the action of mechanical protectives. We supply a few snails in crystallising glasses with uninjured leaves of Symphytum officinale or Boraya officinalis. At the same time they are supplied with leaves of these plants which have been deprived by means of a sharp knife of the rough pointed hairs covering their surface. The latter leaves are readily eaten by the snails; they hardly attack the uninjured leaves at all.

Leaves of Arum maculatum are not eaten by even very hungry snails. The leaves are mechanically protected by raphides, which at once pierce the mouth organs of the snails when they gnaw the leaves, and so produce a highly unpleasant sensation. If we chew small fragments of Arum leaf, we perceive an intense burning taste, which is due to the raphides. Expressed unfiltered Arum juice produces the same sensation when placed on the tongue, while the sap freed from the raphides by filtration has only a sweetish taste.

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[1] See Stahl, *Pflanzen und Schnecken*, Jena, 1888.

V. TRANSLOCATION OF PLASTIC SUBSTANCES IN PLANTS.

138. Experiments with Germinating Pollen Grains.

The observations and experiments which we have made on the behaviour of nitrogenous and non-nitrogenous substances in plants have already made us acquainted with a large and varied range of facts bearing on the migration of substances in the vegetable organism. We will now proceed to treat the subject of translocation more fully. We first select for examination pollen grains.

We first prepare a small moist chamber, by cutting a frame out of not too thick cardboard, making the hole in the middle somewhat smaller than the cover-glass to be employed. After being thoroughly soaked in water, this frame is laid on a slide. We now place on the cover-glass a drop of the fluid in which the pollen grains are to be germinated, add the pollen, taken from ripe anthers, and then with a rapid movement reverse the cover-glass. It is laid on the cardboard frame with the drop downwards, and the germination of the pollen grains can now proceed in the hanging drop. We have only to take care that the chamber is kept well supplied with water.

The pollen grains of Alliums, of Tulipa Gesneriana, and of Narcissus poeticus germinate very readily, according to Strasburger, when they are laid in a 3 per cent. solution of cane-sugar in spring water, and I have myself obtained very beautiful results with the pollen of Allium Victoriale. I transferred some pollen grains to a hanging drop of spring water, and others to a 3 per cent. solution of sugar. At a temperature of 18° C., or 19° C., and in darkness, a development of pollen tubes was already observable in the course of two hours, and two hours later the pollen tubes had grown considerably. In the sugar solution a larger number of pollen grains germinated, and longer pollen tubes were formed than in the spring water. That the germination of the pollen grains must be associated with a transference of material is at once apparent, since, in the development of the pollen tubes protoplasm of course and reserve substances pass over from the pollen grains into the pollen tubes. Since the formation of pollen tubes takes place better in the solution of sugar than in water, it is probable that sugar absorbed from outside can be utilised as food by germinating pollen grains.

139. Experiments with Leaves.

The starch formed in the leaves by assimilation is only employed to a very small extent for the development of the leaf itself. The bulk of the starch leaves the leaf; it travels thence into other organs of the plant, in order to render possible their development. The starch in many cases undoubtedly passes into solution as glucose, which is formed by the action on the starch of diastatic ferments present in the leaves (see 112).

Leaves of Tropæolum, Solanum or Cucurbita are cut off in the evening of a warm summer day. We boil them with water, treat with alcohol in order to remove the chlorophyll, and lay a few of them in Iodine solution (see 14) to prove the presence of large quantities of starch in their cells. The rest of the leaves after treatment with alcohol are washed with water and left for a few hours in a freshly prepared extract of malt at a temperature of 45° C. If we now lay the leaves in Iodine solution, they give no starch reaction, or at most only a weak one, from which it follows that diastase can bring about the solution of the starch developed by assimilation in the cells of the leaf. This process of solution often goes on with quite remarkable rapidity.

We cut in the evening of a very warm summer day in June or July a few leaves of very vigorous plants of Solanum, Nicotiana, Atropa, Cucurbita, or Phaseolus, growing in the open, and at once examine them macroscopically for starch by the method described in 14. They are found to be very rich in starch. If the next morning at sunrise we again remove a few leaves from the plants, and test them, we find no starch in their cells if the night was warm; it has been dissolved during the night, and has travelled from the leaves into other organs.

The following investigation which I made with vigorous pot plants of Tropæolum majus is very instructive. We first make sure by macroscopic tests that the leaves of the plants contain large quantities of starch. A few leaves are cut off, placed under a bell-glass on a moist surface, and screened from the light. The plants themselves are then likewise placed in the dark. After some time (in my investigations, which were made at a temperature of only 12°–15° C., at the end of five days) we test macroscopically for starch in the cut leaves, and also in leaves which were not separated from the parent plant. The latter contain starch only in the nerves, while the cut ones are still more or less

rich in starch. These last could not get rid of their carbohydrates in darkness, being, unlike those left on the plant, unconnected with other organs.

If we apply macroscopic tests to leaves of Impatiens parviflora (this plant often grows wild with us, or it may easily be developed in a somewhat shady place in the garden, from seed), we shall find starch in large quantities in plants which have been exposed to normal conditions of environment. It appears, however (as I have satisfied myself), that the nerves are poor in starch, as compared with the mesophyll, and they consequently assume with Iodine solution a yellow or only faintly bluish colour. If plants of Impatiens are grown in pots, and a few leaves cut from them are placed in darkness, we shall find that the leaves left on the plants, and those removed, are alike devoid of starch after forty-eight or seventy-two hours. In this respect, therefore, leaves of Impatiens removed from the parent plant, and kept in darkness, behave differently from leaves of Tropæolum similarly treated. The glucose, which is the product of solution of the starch, and which passes away from leaves left on the plant, can be reconverted by the Tropæolum leaves into starch. Leaves of Impatiens are at most to a small extent capable of effecting this reconversion.

We now prepare a transverse section from the leaf of Impatiens parviflora, and at once see that the mesophyll is differentiated into palisade and spongy parenchyma. The midrib consists, as usually in leaves, of a peripheral layer of elongated cells poor in chlorophyll, and several vascular bundles the bast of which is covered by a starch layer. The layer of elongated cells which encloses the vascular bundles of the thicker and also those of the thinner nerves we may appropriately designate the conducting sheath.

It has already been mentioned that the nerves, especially the thicker nerves, of the leaves of Impatiens developed under normal conditions of environment are, at all events, poor in starch. If pot plants of Impatiens are placed in the dark for twenty-four hours, and a few leaves are then cut off and tested macroscopically for starch, the scarcity of starch in the nerves is still more clearly brought out. The nerves stand out as a yellow network in the blue-tinted mesophyll, which is still fairly rich in amylum. We leave in darkness for forty-eight hours pot plants of Impatiens, and also leaves cut from these plants and placed in air rich in

aqueous vapour under a bell-glass. All the leaves become almost or completely devoid of starch. Microchemical tests for glucose (for method see 115) show, as I have convinced myself, that in the cells of the conducting sheath of the removed leaves much sugar is present, while the corresponding cells of the leaves not cut off contain but little sugar.

We come to the conclusion that the conducting sheath of the nerves is to be regarded as the tissue which effects the removal from the leaves to other organs of the products of assimilation. In many plants, *e.g.* Tropæolum, the product of solution formed from the starch, especially in the cells of the conducting sheath, can easily be converted transitorily into starch. In other plants, *e.g.* Impatiens, this is either not possible or only possible to a limited extent.[1]

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[1] Literature: Sachs, *Arbeiten des botanisches Instituts in Würzburg*, Bd. 3, Heft 1 (very important). Schimper, *Botan. Zeitung*, 1885, No. 47–49.

140. Experiments with Branches.

If in autumn, just after the fall of the leaves has begun, we test for starch in branches or trunks of our trees several years old (using the method indicated in 110), we find it present in the tissues in large quantities, especially in the medullary rays, and in the parenchyma of the wood and cortex. In Quercus and Betula, etc., the pith also contains much starch; in other cases (*e.g.* in Corylus) the pith is devoid of starch.[1] The condition of the trees or shrubs after the fall of the leaves we may designate as that of autumnal starch maximum. The tissues of their stems are then stocked with very copious quantities of reserve materials which the leaves have produced. In many trees, however, certain elements of the wood and cortex contain in autumn (and also in summer) glucose as well as starch, a fact which is of especial importance. To detect the glucose we use A. Fischer's method. Pieces of branches are split along the middle, laid for five minutes in a concentrated solution of Copper sulphate, washed with water, and then put for two or three minutes in a boiling solution of Sodium Potassium tartrate in soda lye. The necessary sections are now readily prepared. In the glucose-containing elements a precipitate of Cuprous oxide has formed. Generally it is not

necessary to clear the sections; they may, however, be cleared if requisite by glycerine.

If we examine in this way branches several years old of Alnus, Betula, Acer, Syringa, etc., in summer, we find large quantities of sugar in the vessels. The wood fibres and the living wood elements (the parenchymatous cells of the wood and medullary rays) are for the most part free from glucose. In the vessels of the thicker leaf nerves likewise no glucose is present, but it is present in the parenchyma surrounding the nerves, and acting as conducting sheath. At the time of the autumnal starch maximum the distribution of the glucose is nearly the same as in the summer.

When the fall of the leaves has set in and the autumnal starch maximum has come about, solution of the starch in the stems and branches of our trees and shrubs at once begins. This gives rise to the winter starch minimum, which may be complete, say, in December, and lasts till about the beginning of March. In many cases (Salix, Quercus, Corylus, Syringa) the wood, at the time of the winter starch minimum, still contains much starch, while the starch has disappeared from the cortical tissue, and probably has migrated into the deeply lying parts mainly in the form of glucose, which moreover in winter, as in summer, is present in the cortex (starchy trees). If, on the other hand, we examine branches of Tilia or Betula in January, we find starch neither in the wood nor in the cortex (fatty trees). It has been transformed into fat, which can easily be detected in the tissues on treating the sections with alkanet tincture (see 121).

In Tilia, *e.g.*, the medullary rays and inner cortical tissues in particular are fatty in winter, while in autumn they contain much starch. In the pith of Tilia glucose is abundantly present in the winter, besides fat, while particularly the peripheral part of the pith contains in the autumn much starch.

We now continue our experiments by testing branches of Corylus and Tilia, in April, for starch. We again find starch both in the wood and in the cortex. This has clearly been regenerated from fat and glucose. The process of reconversion commences at the beginning of March (in fatty trees at first in the cortex); it continues till about the end of April. At this time the spring starch maximum is attained.

When the leaves begin to unfold, the starch again passes into solution, and in this way is brought about the starch minimum of

the later spring. The solution begins in the young branches, so that these rapidly (perhaps in the course of fourteen days) become free from starch both in the cortex and wood; later it is exhibited in the older branches also. A good object for investigation is Betula.

When the leaves have at last completely unfolded, so that large quantities of carbohydrates are produced, these gradually travel in larger and larger quantities into the stem structures, a process which must naturally be very essentially influenced by the character of the weather. Finally the autumnal starch maximum is reached of which we spoke above.

We make still further the following interesting experiment, in order to show that the process of reconversion of starch from fat or glucose, in the branches of our trees, is very essentially dependent on the temperature to which they are exposed. In winter, at the time of the starch minimum, *i.e.* in December or January, a bough is cut down from a lime tree, brought into the warm room, and left there with its lower end in water. The regeneration of starch begins after a few days, and continues.

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[1] See A. Fischer, *Jahrbücher f. wissenschl. Botanik*, Bd. 22.

141. Ringing Experiments.

For ringing experiments willow shoots are especially suitable. It is best to make the observations in spring, and I obtained particularly good results in investigations with Salis fragilis. The willow shoots, about 200 mm. long and 12 mm. thick, are ringed at their morphologically lower end, the process consisting in the removal of a ring of cortex about 20 mm. broad at a distance of say 40 mm. from the bottom, so that at this place the wood is laid bare. The branch is now suspended in a sufficiently tall glass cylinder by twisting a thread round its upper end, and fastening the thread by means of sealing-wax to a glass plate closing the mouth of the cylinder. The bottom of the cylinder is covered to a depth of a few millimetres with water, into which however the shoots must not be allowed to dip. Moistened strips of blotting-paper, lining the inner side of the cylinder, materially assist in keeping the air in it uniformly moist. In an investigation which I made, a ringed willow branch was left in a glass cylinder in the dark from March 19th till April 21st. The result of the experi-

ment is indicated in the drawing below. The short piece, 45 mm. in length, below the ring has produced small roots; from the portion above the ring long roots have been put out, and at the upper end shoots also. The reason that only short roots are produced in the short length below the ring is to be sought in the fact that here an insufficient quantity of plastic material is available. The small quantity of nitrogenous and non-nitrogenous formative material in the part of the shoot below the ring is rapidly used up. Non-nitrogenous substances may, it is true, still stream to it, for it can be readily determined (for method see 110) that, especially in the peripheral region of the wood, much starch is present, as I satisfied myself, *e.g.*, in February, using a branch of Salix fragilis, 6 mm. in diameter. But the ringing stops the conduction of proteids which, as the ringing experiments themselves and other observations teach (see 143), is chiefly effected by the elements of the soft bast. Naturally the formation of roots below the ring is more vigorous the higher the place on the branch from which the ring of cortex is removed. On the other hand, no formation of roots at all took below the ring when the short piece at the lower end of the branch was only 20 mm. in length. If we do not remove a complete ring, but

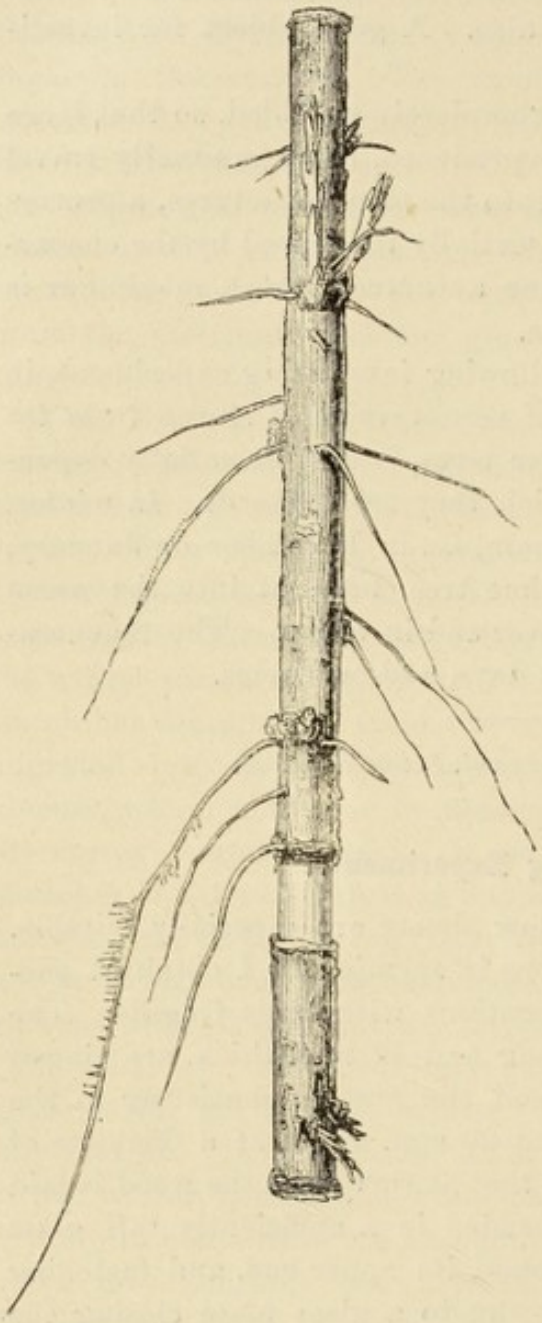

FIG. 116.—Ringed branch of Salix fragilis, in the upper part of which vigorous roots and shoots have developed.

allow a vertical strip of cortex to remain as a bridge between the short lower and the long upper segments of the shoot, there is a comparatively luxuriant formation of roots in the lower part, which is due to the fact that not inconsiderable quantities of proteid can cross the bridge.

If we examine under the microscope transverse sections of willow shoots, we shall easily make out that the wood of the fibro-vascular bundles on its inner side directly abuts on the pith. Bast is only present between the cortex proper and the outer side of the wood, and therefore the conduction of proteid must be interrupted if the ringing extends right through to the wood.

Ringing experiments with Mirabilis Jalapa or Nerium Oleander yield results very different from those obtained with willow shoots, and generally with shoots possessing the typical dicotyledonous structure. And, in fact, the anatomical structure of the stems of these plants is very peculiar.

Transverse sections of Mirabilis stems about 4 mm. in thickness show under the microscope the epidermis, the primary cortex with its external ring of collenchyma, and, in particular, a ring consisting of strongly lignified cells (sclerenchymatous fibres) and intercalated vascular bundles, and, finally, the central part of the stem. This latter consists of medullary tissue, whose cells I still found to contain large quantities of starch at the end of October, when the leaves of the plants had been killed by a night frost, and, distributed in the ground tissue, vascular bundles with distinct bast and wood. These central vascular bundles are not reached at all by ringing, and consequently in this case the removal of the ring of a cortex does not materially interrupt the paths of conduction either of the non-nitrogenous or of the nitrogenous plastic substances.

We select a vigorous, very leafy shoot of Nerium Oleander, remove a ring of cortex at a distance of about 20 mm. from its lower end. We then pass the shoot through the cork closing a vessel filled with water, fixing it in the hole of the cork in such a way that it dips into the water to a depth of about 80 mm.

If the temperature is high enough, and the air not too dry (it is advisable to keep the shoots in the hothouse), many roots, after some time, break out from the parts of the stem, above the ring, which are in the water. After a time a fairly large number of roots also develop at the base of the shoot, *i.e.* below the ring. Nerium shoots thus behave quite differently to willow shoots, and

the cause of this is to be sought in the anatomical structure of the stem. In Salix soft bast elements occur only at the periphery of the vascular bundles. Nerium possesses soft bast not only on the outside, but also on the inside of the vascular bundles, as is easily made out microscopically by examining delicate transverse sections. In Nerium, therefore, the path for the conduction of proteids is by no means completely blocked by ringing, while in Salix it is. In Nerium considerable quantities of non-nitrogenous and also nitrogenous plastic material can stream to the parts of the stem lying below the ring, and for this reason a fairly large formation of roots is possible in this lower portion of the stem.[1] *

As regards the migration of non-nitrogenous bodies in trees and shrubs under normal conditions of environment, there is no doubt, according to the investigations of Th. Hartig, Sachs, and A. Fischer,[2] that the carbohydrates formed in the leaves are translocated almost exclusively in the parenchyma of the cortex, while the upward movement of the carbohydrates in the spring, when the buds are bursting, takes place in the glucose-containing vessels of the wood.

A several-years-old branch of Betula, the lower end of which must be without branches and leaves, is ringed at the beginning of June about 10 cm. above its point of insertion, without being removed from the parent plant. The exposed wood is smeared with grafting wax. At the commencement of August we cut the branch and test suitable transverse sections for starch. It is often sufficient to examine macroscopically, by moistening transverse slices with Iodine solution. Above the ring the wood and cortex prove to be extremely rich in starch. The wood at the ring is very poor in starch; so also are the wood and cortex below the ring. The carbohydrates clearly could not pass the ring, because here there was no cortex. In the uninjured branch, on the contrary, they move downwards in the cortical parenchyma, and thence distribute themselves to the wood, the medullary rays, and the pith, where they are deposited in the form of starch.

The fact that the carbohydrates move upwards in the wood at the time of the bursting of the buds is determined by the

* Proteids can, however, in many cases be translocated also in the wood. See Strasburger, *Bau und Verrichtung der Leitungsbahnen*, 1891, pp. 900 and 901. The translocation of amides, etc., takes place in the parenchyma.

following experiment. At the end of January we cut a Syringa shoot provided with two one-year-old branches, put it with its base in water, and ring it just below the point at which it forks. In the warm room the buds unfold in the course of a fortnight or three weeks. The wood laid bare is to be covered with grafting wax. At the commencement of the experiment the wood of the twig contains much starch. When the buds have unfolded, the starch has almost completely disappeared, not only from the branches, but also from the wood at the level of the ring, and from the wood of the two-year-old portion of the branch below the ring. The substance of the starch has been sent upwards in the vessels of the xylem in the form of glucose.

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[1] Literature: Hanstein in Pringsheim's *Jahrbücher*, Bd. 2; Sachs, *Flora*, 1863, p. 33.

[2] See A. Fischer, *Jahrbücher*, Bd. 22, pp. 137 and 142.

142. The Starch and Sugar Sheaths and their Functions in Connection with Translocation.

Many plants are characterised by the possession of a starch sheath, and we may conveniently observe such a sheath if we prepare transverse sections from the stems of, *e.g.*, bean plants, which have developed in darkness till the first internode has elongated considerably. Epidermis, cortex, pith, and vascular bundles are easily distinguished. The circle of vascular bundles is surrounded on the outside, *i.e.* on the bast side, by a layer of cells whose elements are smaller than those of the cortex, and this is the starch sheath. We find large quantities of starch in the cells of this bundle sheath, a fact which has led to the conclusion that the translocation of carbohydrates takes place especially in the starch sheath. Nevertheless, various facts are not in unison with such a view. We prepare in July transverse sections from the lower part of the stem of a vigorous plant of Phaseolus grown in the open. Each vascular bundle is furnished on the outside with a strongly developed layer of bast fibres, and we can easily make out, by microscopic examination, that large quantities of starch are present in the parenchyma both of cortex and pith. The cells of the starch sheath, on the other hand, are, as I have satisfied myself, very poor in starch, or contain no starch grains at all. From this it follows that translocation of

starch undoubtedly takes place chiefly in the cortex and pith. The starch sheath only contains much starch when the elements of the bast fibre layer of the bundles are not yet fully developed. With advancing development of these the starch gradually disappears from the cells of the starch sheath, being employed in building up the thick-walled bast elements.[1]

The fact has already been mentioned that many plants have the power of reconverting transitorily into starch, along the channels of conduction (the leaf-nerves), the carbohydrates which have migrated from the mesophyll of the leaves. Other plants can effect this only to a small extent, and hence we find their leaf-nerves filled not with starch but with glucose. We prepare transverse sections from the lower part of the lamina and the upper part of the leaf-stalk of a mature turnip leaf. The starch formed in the blade by assimilation travels under normal conditions through the nerves and the leaf-stalk into the root, rendering possible its development. By micro-chemical tests, however, we find only very small quantities of starch in the parenchyma which surrounds the vascular bundles of the nerves and of the leaf-stalk, whereas we find very large quantities of glucose, and we may therefore designate the tissue conducting the carbohydrate a conducting sheath, and in particular a sugar sheath.[2]

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[1] See H. Heine, *Berichte der Deutschen botan. Gesellschaft*, Bd. 3, Heft 5.
[2] See H. de Vries, *Landwirthschl. Jahrbücher*, Bd. 8, p. 445.

143. The Sieve Tubes and their Functions in Connection with Translocation.

If we cut through the stem of a plant of Cucurbita, a considerable quantity of a mucilaginous fluid springs from the cut surface. On consideration of its quantity, it is at once clear that the sap is forcibly driven out under the influence of pressure, and in fact causes for such pressure effects are to be found in the organism, as we shall see below. We will first examine the sap.

We cut through the stem of a Cucurbita, *e.g.* C. Pepo (I used C. minensis). We now touch the cut surface with a small piece of red litmus paper, and find to our surprise that it becomes blue. At all events, therefore, a large part of the sap flowing from the stem has a comparatively strong alkaline reaction, whereas most plants when they have been wounded yield a sap acid in reaction,

and consequently colouring blue litmus red. If we again touch our cut surface with red litmus paper, we shall soon find that the whole surface of the paper touching it is no longer turned blue, but only particular parts, those namely which come into contact with the vascular bundles. If we now touch the cut surface with blue litmus paper, this becomes red except in certain places. Directly after cutting the stem of Cucurbita there exudes a mixture of sap preponderatingly alkaline in reaction. Proceeding, however, as above, we readily make out that the sap of the parenchyma in Cucurbita, as in other plants, is acid in reaction, while that of certain tissues of the fibro-vascular bundles, viz. those of the soft bast, have an alkaline reaction. In other plants the conditions are similar, but it is not so easy to determine them with certainty.[1]

We now prepare a transverse section from the hypocotyl of Cucurbita Pepo, using alcohol material. In most plants soft bast occurs only on the outside of the vascular bundles, but here it is to be found both on the outside and the inside of the wood of the bundles. If we test for proteids in the manner given in 94, it is found that the elements of the soft bast contain large quantities of proteid substances. The alkaline mucilage, rich in proteid, which escapes on cutting the Cucurbita stems, is present in particularly large quantities in the sieve tubes of the soft bast, characteristic elongated elements which are divided by transverse walls (the sieve plates), pierced by numerous pores. In the sieve tubes parietal protoplasm is present, and they are filled with an alkaline mucilage rich in proteid, which by means of the sieve pores can pass from one member of the sieve tube to another. And in fact such a movement of the mucilage must take place in the uninjured plant, from the same causes as bring about the escape of the mucilage when the plants are injured. The sieve tubes, viz., are subjected to the pressure of the turgescent parenchyma in their neighbourhood. Hence their contents can be passed on to places of less pressure, especially to the very young parts of the plant, and we see therefore that the sieve tubes function as organs for the translocation of proteids, which is in complete harmony with the results obtained from the ringing experiments.

The circulating proteid in the mucilage of the sieve tubes moves *en masse*, and can be transported from one place in the plant to others often far removed. We must however make our-

selves acquainted somewhat more accurately with the structure of sieve tubes, especially with that of their sieve plates.

We prepare transverse sections from a stem of Cucurbita Pepo (alcohol material) 10 mm. in thickness. Under a low power we observe the epidermis, the interrupted collenchyma, the cortex, the ring of sclerenchyma, and the vascular bundles grouped in a double circle. These are made up of a wood portion with very wide vessels, and an inner as well as an outer bast portion. The inner bast or sieve region clasps the inside of the wood or vascular region in a crescent-like manner. For more accurate examination

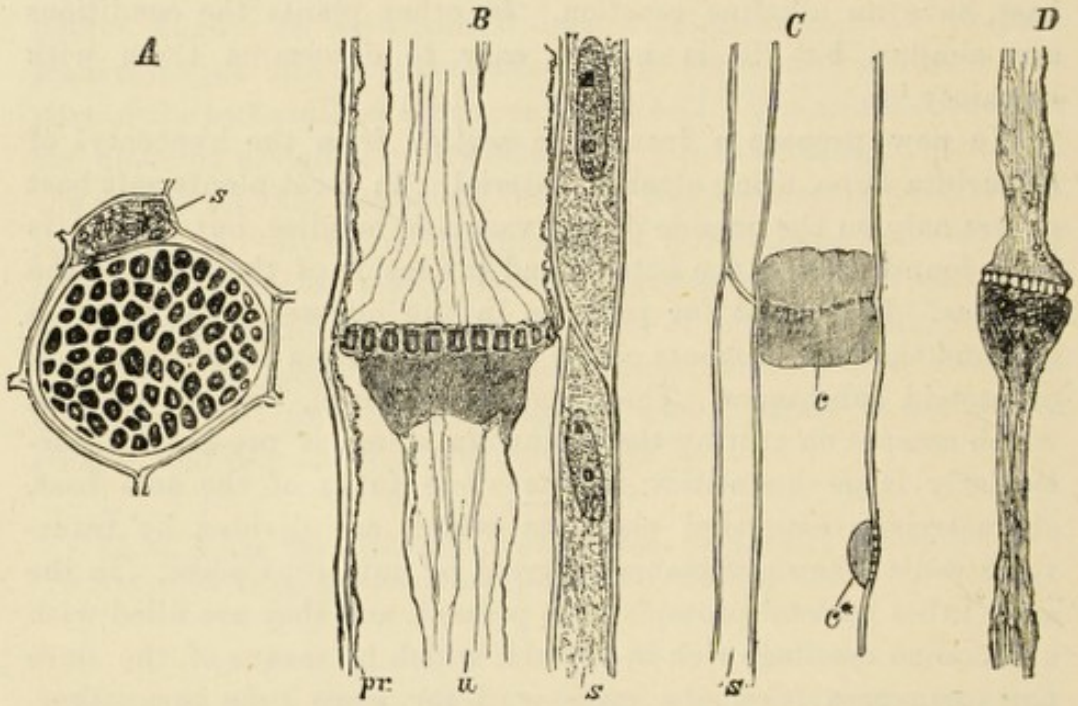

FIG. 117.—Cucurbita Pepo. Portions of sieve tubes from alcohol material. *A*, in transverse section; *B–D*, in longitudinal section. *A*, a sieve plate seen from above; *B* and *C*, two elements seen from the side; *D*, the connected contents of two elements after treatment with Sulphuric acid; *s*, companion cells; *u*, strand of mucilage; *pr*, protoplasmic tube; *c*, plate of callus; *c**, small plate of callus on one side of a lateral sieve area. Magn. 540. (After Strasburger.)

under higher magnification, it is well, according to Strasburger, to lay the sections in aniline blue for a short time, and then mount them in a drop of glycerine. The tissue of the inner and outer bast is made up of wide sieve tubes, of their companion cells with contents stained blue (see Fig. 117), and finally of cambiform cells. The perforated sieve plates are easily recognised where the sections have passed through them.[2]

[1] See Sachs, *Botan. Zeitung*, 1862.

[2] See Wilhelm, *Beiträge zur Kenntniss des Siebröhrenapparates dicotyler Pflanzen*, Leipzig, 1880, and Fischer, *Untersuchungen über das Siebröhren system der Cucurbitaceen*, Berlin, 1884.

144. Latex.

Many plants, as is known, contain latex. If we cut a Euphorbia, for example, a white milky juice often escapes from the wound in large quantities (especially if we experiment with the Cactus-like Euphorbias).

Clearly the contents of the latex reservoirs must stand at a not inconsiderable pressure, exerted by the turgescent cells of the parenchyma in their neighbourhood, because otherwise such large quantities of fluid could not flow from the wound as is actually the case when laticiferous plants are cut.

The question as to the physiological function of latices is still unsolved. I cannot escape the impression that latices are *inter alia* of significance in nutrition; the experiments of Faivres are also in favour of this. Latex may, however, be of special importance as a means of protection to the plants.

In the watery fluid of latices, as in the serum of the milk of animals, there are suspended numerous small solid particles, so that most latices appear white in colour. The number of these solid particles, however, varies considerably from time to time, and according to the origin of the latex. If we take a drop of latex from the stems or the leaves of a fig, and mount it on a slide without addition of water, we can easily make out by examination under high magnification that the number of particles in suspension is comparatively small. The latex of the Euphorbias, and that of Ficus elastica are usually found to be much richer in solid constituents.

In the watery fluid of latices there occur in solution mineral substances, sugar, proteids, and sometimes also pepsin (see 96), etc. The suspended particles very often consist mainly of indiarubber. Many latices, however, also contain fat or starch grains.

If latex from a Euphorbia is mixed on the slide with a little water or alcohol, the latex coagulates. Under the microscope it is seen that the constituents which were originally uniformly distributed in the latex have gathered themselves together into larger masses.

The latex reservoirs of plants are of very different kinds. A very favourable object for study is found in the root of Scorzonera hispanica (black root). We employ alcohol material, and after removing the superficial layers of cortex prepare tangential longitudinal sections. The latex reservoirs, readily recognised by their contents, appear in our preparations as elongated vessels, frequently anastomosing with one another, and traversing the small-celled parenchyma.

We further submit to examination stems of Chelidonium majus, using alcohol material. Transverse sections show the epidermis, collenchyma, and green cortical parenchyma. These are followed internally by a closed ring of mechanical tissue, whose elements are strongly thickened. The vascular bundles consist of a bast portion and a wood portion. In the bast, but also in the ground tissue surrounding the fibro-vascular bundles, we perceive elements with brown contents. These are the latex vessels. The latex of Chelidonium has an orange-red colour in the fresh state. The treatment of the material with alcohol has caused the latex to coagulate in the laticiferous vessels.

145. Accumulation of Material.

It is clearly an important fact that some tissue-complexes in the plant serve as channels of conduction, others as places for the deposition of particular substances. We are not at present in a position to give a detailed account of the causes of the phenomena. It is only possible to do so in a general way, and I have already discussed these matters in my *Lehrbuch der Pflanzenphysiologie.*

If, *e.g.*, an accumulation of starch is to take place in the tissues of the reserve receptacles, causes must be at work in their cells, which bring about the precipitation, in the form of starch grains, of the non-nitrogenous material conveyed thither. Similar causes must also be operating in the transitory formation of starch in the cells of the tissues which act as channels for the conduction of carbohydrates. The accumulation of starch can only take place in cells in which starch-formers occur, and their activity is the necessary condition for a continuance of the current of dissolved carbohydrate. The results of the following experiments serve to afford us an idea of the nature of the process of accumulation.

We fill a beaker with dilute Copper sulphate solution. In this we suspend a glass tube about 6 cm. long and wide, closed at both ends with parchment paper, and filled with water, into which a spiral of zinc has been introduced. The solution of Copper sulphate penetrates into the tube, and distributes itself in the water; but when it comes into contact with the Zinc it is decomposed, and forms soluble Zinc sulphate, while the Zinc becomes coated with a gradually thickening crust of metallic Copper and Copper oxide. The accumulation of Copper in the tube is thus easy to prove.

That dissolved substances can be separated from their solvents, and stored up by bodies capable of imbibition, may be demonstrated as follows:—We treat water with a few drops of an alcoholic solution of Iodine, so that the fluid assumes a yellowish colour, and add to it some wheat starch. This last takes up the Iodine. It consequently becomes blue in colour, while the fluid is rapidly decolorised. We further provide a glass funnel with six or eight filter papers, placed one within the other, and pour on them a dilute aqueous solution of methyl violet. The paper completely arrests the pigment. A colourless fluid runs from the filter.

SECOND PART.

Physiology of Growth and Movements Resulting from Irritability.

FOURTH SECTION.

Movements of Growth.

I. CHARACTERISTICS OF GROWING PLANT STRUCTURES, AND MOVEMENTS OF GROWTH DEPENDENT ON INTERNAL CAUSES.

146. The Extensibility and Elasticity of Growing Plant Structures.

It is of great significance, in connection with the theory of growth, that growing plant structures are highly extensible and elastic. We shall return to this again in detail; here it is only proposed to establish the fact in a general way.[1]

For investigation we select perfectly fresh pieces of stem cut from plants of Aristolochia Sipho or Sambucus nigra. At the upper and lower end of a young internode and of the next older one we make fine lines with Indian ink, as points of reference, take the object with both hands, and, laying it against a millimetre scale, stretch it as strongly as may be without risk of breaking it. It is now easy to prove that the younger internodes are much more extensible than the older; and I found, *e.g.*, that the extensibility of a young internode of Aristolochia Sipho, 50 mm. long, amounted to 9 per cent. If we leave the shoots to

themselves after they have been stretched, they shorten again more or less; their tissue is therefore elastic. Since, however, they do not completely resume their original length, but after vigorous stretching remain permanently longer, they must be considered incompletely elastic.

Perfectly fresh, straight internodes of Vitis or Aristolochia, about 6 mm. thick, are bent with both hands over a card, marked with concentric circles, till the axis of the stem coincides with one of the circles. The known radius of this circle we note as the radius of curvature of the bent internode. If we leave the stem to itself, it does not become straight again, but remains somewhat considerably bent; and we may again easily determine its radius of curvature. Growing plant structures are therefore flexible. They have elasticity of flexion, but the elasticity is not perfect.

If we select a straight, actively elongating shoot, with a stick give it a blow or several blows in succession at the lower end, where the growth in length is already completed, the curvature produced in the region struck travels in the form of a wave right up to the free end. This consequently appears bent, and the concavity always lies on the side from which the blow came. This curvature, resulting from a blow or concussion, which I observed especially beautifully in experiments with shoots of Vitis and shoots of Lonicera tatarica, owes its origin, as is more fully explained in my *Lehrbuch der Pflanzenphysiologie*, to the flexibility and incomplete elasticity of the structures.

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[1] See Sachs, *Textbook of Botany*.

147. Relations between Turgidity Growth and Extensibility in Plants.

For our purpose it is, first of all, indispensable to determine the relative rate of growth in the successive partial zones of a plant structure. We experiment with young flower-stalks of Butomus umbellatus, Plantago media, and Papaver, cut from the plants, or with undetached epicotyls of Phaseolus multiflorus, which have developed in darkness to a length of a few centimetres. By means of Indian ink we make fine lines on the objects, at distances of every 10 or 20 mm., to serve as marks, and so divide them into a series of partial zones. The placing of the marks necessitates some care; it is to be done in the manner

given in 59 and 148.* The detached flower-stalks are now placed vertically in a cylinder filled with spring water, so that they are completely covered, while the bean-stalks are not yet cut off. After twelve or twenty-four hours we determine the distances between the marks; and we shall find that these are no longer 10 or 20 mm., but have become greater. This is due to the growth which has taken place. It can now be easily determined, and this is of special interest, that the growth of the individual partial zones is by no means the same. The most rapid growth has taken place either in the very youngest partial zone (so I found in harmony with H. de Vries, using for investigation a young flower-stalk of Plantago, divided into partial zones 20 mm. in length), or in one of the youngest zones (*e.g.* the third). I found this to be the case in experiments with the epicotyl of Phaseolus seedlings grown in sawdust in the dark. The epicotyl was 70 mm. in length, and the marks were made at distances of only 5 mm. from each other. In the course of forty-eight hours at 15° C., the youngest partial zone increased in length by 1 mm., the second by 3 mm., the third by 8 mm., the fourth by 6 mm., the fifth by 5 mm., the sixth by 3 mm., and the seventh by 1 mm. It is always found that the rate of growth of the cells gradually diminishes with advancing age, until finally their growth completely comes to an end.

After determining the distribution of the rate of growth in the objects investigated, we plasmolyse them (see 59) by placing them in a 10 per cent. solution of common salt or Potassium nitrate. Pieces of stem 2–3 mm. thick may be at once placed in the salt solution; thicker pieces must first be halved. After a shorter or longer time (three to twelve hours), plasmolysis is complete. The partial zones have become shorter owing to loss of turgidity; and if we calculate the shortening with reference to the original length of the zones (5, 10 or 20 mm., as the case may be), it appears that it is greatest in extent, roughly or exactly, in the regions where the most vigorous growth took place. There is a clear relation between the turgor-extension of the cells of the different partial zones and their rate of growth, a result which leads to the view that the rate of surface growth of cells is dependent upon the magnitude of their turgor-extension. This last is deter-

* If it is required to place ink-marks on curved plant structures—*e.g.* the epicotyls of Phaseolus—it can be done by means of a strip of paper divided into millimetres.

mined by the magnitude of the osmotic pressure [*Turgorkraft*], and by the amount of resistance offered by the stretched cell layers (protoplasm, cell-wall). This resistance depends, among other things, upon the extensibility of the stretched cell layers, so that special interest attaches to the exact determination of this extensibility.

We use for investigation, *e.g.*, an epicotyl of Phaseolus, 30 mm. in length, which has been divided into partial zones 5 mm. in length. We determine its rate of growth, and then plasmolyse it. The limp stem is carefully laid upon a sheet of cork, and covered at its upper end with a small sheet of cork, the two sheets being then clamped together by means of a binding screw. To the older end of the stem we fasten a piece of thread with a loop. This is pulled till the desired extension of the object is obtained, and then fixed to the cork by means of a needle. The stem is only stretched till it is artificially brought back to the length which it possessed before being plasmolysed. By means of a millimetre scale we determine the amount of extension of the individual partial zones, and express the results in terms of the original equal lengths of the zones (5 mm.). It is thus proved that the extensibility of the tissue in the younger regions of the structure is considerably greater than in the older ones. A relation is therefore established between the rate of growth, the amount of turgor-extension, and the extensibility of the tissue in the different partial zones.[1] Wortmann, *e.g.*, has, in experiments with a young bean epicotyl, which he had divided into six zones originally 5 mm. each in length, determined the following values for the growth of the partial zones in twenty-four hours, for the shortening in salt solution, and for the increase in length by stretching.

Zone.	Lengths after growth for 24 hrs. at 25° C. Total length =64·5 mm.	Partial increments in 24 hrs.	Lengths after immersion for 10 hrs. in the solution. Total length =60·5 mm.	Lengths after stretching the whole up to the original length of 64·5 mm.	Whence actual extensions.	And therefore extensions per equal lengths (10 mm.).
I	8·5	3·5	7·5	8·5	1·0	1·33
II	17·5	12·5	16·0	18·0	2·0	1·25
III	17·5	12·5	16·5	17·5	1·0	0·60
IV	9.0	4·0	8·5	8·5	0·0	0·00
V	6·0	1·0	6·0	6·0	0·0	0·00
VI	6·0	1·0	6·0	6·0	0·0	0·00

These and other observations always showed, what is not in complete harmony with the results obtained by H. de Vries, that the zone of greatest extensibility lies in the youngest region of the structure investigated, and is not coincident with the zone of most vigorous growth. In the youngest zones, however, as Wortmann determined (*Bot. Zeit.*, 1889, p. 250), the osmotic pressure is relatively small; and hence, in spite of the great extensibility of their membranes, they still do not grow very energetically. The maximum of growth falls in a zone whose cells are still very extensible, and develop high osmotic pressure (hence here also the turgor-extension of the cells is greatest); while in the older zones the growth is again slower, notwithstanding the fact that the osmotic pressure of the cells keeps high, because the extensibility of the membranes falls off very considerably.

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[1] The fundamental ideas of our current theory of growth have been developed by Sachs. See my *Lehrbuch der Pflanzenphysiologie*, p. 213. Respecting the experiments indicated, the student is referred to the work of H. de Vries, *Ueber mechanische Ursachen der Zellstreckung*, Halle, 1877. See also Wortmann, *Botan. Zeitung*, 1889.

148. The Contraction of Roots.

In many plants careful observation shows that while the seedlings expand their cotyledons above ground, and the plumule projects more or less out of the soil, the points of insertion of the cotyledons and of the leaves developed from the bud are at a later stage concealed in the soil. This subsequent dragging into the soil of the points of insertion of the leaf structures can only be caused by contraction of the root; and, in fact, the occurrence of such a phenomenon has been definitely determined by H. de Vries.[1] The contraction, whose biological significance is to be sought in the protection which it affords to the buds in the soil, is due to peculiarities in the growth of the roots. In the cells of the parenchymatous tissue of the roots occurs energetic turgor. This must be the case before growth can take place at all. But since in the somewhat older roots the extensibility of the membranes of the cells is greater in the transverse direction than in a direction parallel to the long axis of the roots, the extension of the cells due to turgor is actually greater in the former direction than in the latter, and contraction of the organs must

consequently take place. The shortening is then gradually fixed by growth. It is now of special interest to study accurately this contraction of the root resulting from turgor, since it is the indispensable condition for the subsequent shortening, to be rendered permanent and irreversible by growth.

We sow seeds of Carum carvi in good garden earth in the open, in summer, and let the plants grow till they are two to three months old. I sowed at the end of July, and used the material for investigation at the end of October. When the experiments are to be made we remove the plants from the soil, at once cut off the tops, so as to provide against excessive loss of water from the roots owing to transpiration by the leaves, bring the roots into the laboratory, and after washing and drying them remove their secondary roots and the thin terminal portion. It is now necessary to provide the roots with ink marks, and to do this we lay each of them in turn on a sheet of cork along one half of which is fastened a second sheet of cork, whose thickness is about the same as that of the root to be operated upon. The root is placed against the edge of the upper sheet of cork, and fixed there by means of needles, which are stuck into the lower sheet close up to the root. The ink lines are finally painted on at definite intervals with the help of a brush and a millimetre scale. In my experiments with Carum roots, which at the upper end, *i.e.* at the morphological base, were 6–9 mm. in thickness, the distance between the two marks was 70–100 mm. The roots are next placed in flat glass dishes filled with water.* If now, after a certain time, we measure the distance of the marks from one another, at intervals of, say, 2, 4, 24, 2×24, and 4×24 hours, we find that they get nearer and nearer, till finally no further contraction takes place. The extent of the contraction is considerable. In different cases which I observed it reached at the end of twenty-four hours 2·5 to 4·0 per cent.

If we dry the contracted roots and plasmolyse then by immersion in common salt or Potassium nitrate solution, it is found that while becoming flaccid they have, even after a few hours, elongated considerably. This elongation, however, stands in the closest relation with the contraction accompanying the increased turgor of their root cells due to absorption of water.

* It is advisable before placing the marked roots in water to let them stand for a few minutes in moist air, so as to make sure of the ink adhering.

When roots lie in water their total volume naturally increases, notwithstanding the fact that they shorten; and the volume of their constituent cells likewise increases. Extension of the cells takes place only in a direction at right angles to the axis of the root; the root becomes thicker, and we make the following observations in order to determine this extension:—

We prepare transverse sections of the Carum roots ·5 mm. in thickness, isolate a median portion of each by two parallel cuts, and indicate its length on paper under slight magnification (about 10 diameters) by means of a camera lucida. The sections are now quickly put into water. If we again mark their length on paper after about an hour, it is found on comparison that the strips have increased in length. Dividing the lengths observed by 10 (if we have worked with a magnification of 10 diameters), we obtain an absolute value for the lengths of the strips before and after the absorption of water.

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[1] See H. de Vries, *Landwirthschl. Jahrbücher*, Bd. 9, p. 37.

149. Longitudinal Tension.

To determine that in many plant structures longitudinal tensions occur, straight internodes, or portions of such, are laid on a piece of thick cardboard, on which fine lines have been drawn, and the length of each is indicated by two marks made with a very sharp lead pencil. Then with a sharp razor we remove strips of the different tissues (epidermis, generally with the collenchyma attached, cortex, wood, and pith, which we free from wood by longitudinal cuts) the full length of the internodes, and without dragging them, lay the isolated strips on the cardboard, and mark off their length as before. We can now, by means of a millimetre scale, determine the lengths of the uninjured internodes and of the tissue isolated from them. We may employ for the observations vigorously growing internodes, about 50 mm. long, from Sambucus nigra, Nicotiana Tabacum, Vitis vinifera, or Helianthus tuberosus. It is always found that the length of the isolated strips of tissue increases from outside inwards, the pith isolated from actively growing internodes being generally much longer, and the isolated epidermis shorter, than the uninjured internode, while a strip of tissue taken from the region between the epidermis and pith is exactly, or nearly, the same length as

the intact structures. The pith is therefore in a state of great positive or active strain (compression), the epidermis in a state of negative or passive strain (tension).

If the length of the uninjured internode is taken as 100, and the change in length of the epidermis and pith be expressed in percentages, we obtain a value (not, it is true, an absolutely exact one) for the intensity of the tension in the uninjured structure. For example, if the total length of an internode used for experiment is 50 mm., the length of the isolated epidermis 49, and that of the isolated pith 54, the tension in the uninjured structure would be expressed by the number 10. As a matter of fact we frequently obtain such numbers in experiments, *e.g.*, with internodes of Sambucus nigra.

It is instructive to determine the tension in the manner above described in the successive internodes of a shoot. If we always refer the change in length of the isolated strips of epidermis and pith to 100, we obtain comparable numbers, and it is then brought out that the tension in the youngest internodes is small, while it rises to a considerable value in those somewhat older, becoming much less again in the still older internodes. Shoots of Sambucus nigra form particularly good material for such investigations.

With reference to the causes of the longitudinal tension in the internodes, it is to be observed that its origin must primarily be traced back to the strong turgescence of the cells of the pith. The cells of the pith are able to absorb very large quantities of water. The pith consequently endeavours to extend as much as possible, and strives to stretch the extensible peripheral tissues. These, however, are not only extensible, but at the same time elastic, and strive on their part to compress the pith. The high turgescence of the cells of the pith further brings about in them specially vigorous growth, a condition which must still further intensify the tension in the internodes. When the pith, with advancing age of the stem, loses its water, and ceases to grow, the longitudinal tension also disappears. In place of it, however, is exhibited, in connection with the vigorous growth in thickness which now goes on, the transverse tension which we have to discuss in 150.

That the pith does actually possess the power of taking up without difficulty considerable quantities of water, may easily be demonstrated in lecture. In flower stalks of Taraxacum officinale, microscopic examination of a transverse section teaches that

epidermis, collenchyma, green tissue, and pith parenchyma follow each other in succession from without inwards; in the ground tissue lie the fibro-vascular bundles. If we split a young flower-stalk longitudinally, and lay the pieces in water, they rapidly roll up into a spiral under the eye of the observer, the pith side being convex (see Fig. 118). The medullary tissue rapidly takes up large quantities of water, its cells lengthen, and the structure consequently curls up spirally. Two internodes, as nearly as possible similar to each other, are cut off and examined in the manner above described as regards their tensions. One is investigated immediately after being cut off; the second after it has become somewhat limp through being left in the air. We shall find the difference in length between the isolated epidermis and the isolated pith greater for the first than for the second, which again indicates that the amount of water in the tissues is of great significance in determining the magnitude of the tensions occurring in plant structures.[1]

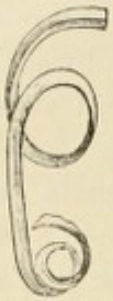

FIG. 118.—Flower scape of Taraxacum officinale, halved longitudinally, which has coiled in consequence of absorption of water.

[1] See Kraus, *Botan. Zeitung*, 1867. Further information of importance in discussing the subject of tension in tissues will be found in my *Lehrbuch der Pflanzenphysiologie*, 1883, p. 229.

150. Transverse Tension.

To determine the existence of transverse tension in any part of a stem structure, we cut out a transverse slice at this place and measure its circumference with a strip of paper, then break the continuity of the peripheral tissues by a perpendicular radial cut and strip off the whole cortex. The isolated ring of cortex is now replaced in its original position without stretching. It is found, however, that the cut surfaces no longer meet together, whence it follows that the cortex must, in the intact structure, be passively or negatively stretched (see Fig. 119). If we measure the distance between the two ends of the divided ring of cortex after it has been replaced, and subtract the result from the length of the circumference of the intact transverse slice, we obtain a number which expresses the length of the ring of cortex after isolation.

The tension can finally be easily expressed in percentage of the original circumference. Suitable material for determining the existence of transverse tension is afforded by Helianthus stems, or five to ten year old trunks or branches of species of Prunus, Pyrus, or Salix. I have, *e.g.*, investigated the tensions in transverse slices, 5 mm. in thickness, from branches of Prunus insititia and from a Salix. Circumference of discs 106 (Prunus), and 132 (Salix), respectively. Distances between cut surfaces, 4·5 and 6 mm. respectively. Tensions, 4·2 and 4·5 per cent. respectively.

If in the manner described we determine the tension simultaneously at different levels in a stem structure,—if, *e.g.*, we take slices from a stem of Helianthus annuus at the base, at the middle, and at the upper end respectively, and then in each case isolate the cortex,—it will be found in general that the transverse tension in the younger parts of the structure is relatively small, but that it augments considerably in the older parts.

High transverse tension in stem structures is associated with the occurrence of rapid growth in thickness in them. When this is proceeding the circumference of the central tissues (especially of the wood) increases more rapidly than that of the peripheral tissues. These consequently become stretched, and shorten on isolation. But the resulting wood is by no means always of the same circumference; it may indeed vary considerably in size, which is easily explained when we consider that the quantity of water of imbibition in the wood at any time exerts a marked influence on its volume. Increase in the quantity of water in the wood must consequently result in increased tension in the stem. If, *e.g.*, we cut slices from a branch of Prunus insititia, determine the tension in some of the slices at once, while the others are first placed in water for twenty-four hours, it is found that the tension is greater in the latter than in the former. In experiments which I made with Prunus, using slices about 15 mm. thick, and about 100 mm. in circumference, the transverse tension increased from 4·5 to 5·5 per cent. when the discs were left in water for twenty-four hours. As regards the relations between the tension and other external

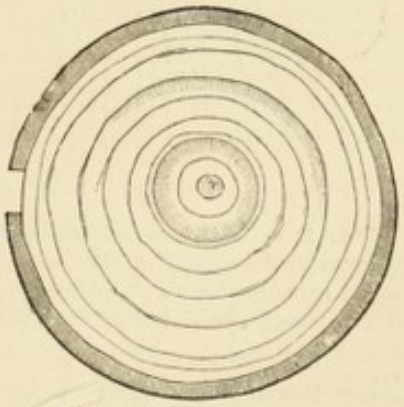

FIG. 119.—Transverse slice of a bough of Prunus. The cortex has first been isolated and then again laid round the wood.

factors (temperature, light), I refer the student to the treatise by G. Kraus in the *Botanische Zeitung*, and to my *Text-Book of Plant Physiology*. In these places also reference is made to the important, though certainly not yet sufficiently studied phenomena of periodicity in tissue tension.

151. The Growing Points and Growth in Length.

The growing points of plant organs are of very different character as regards detail. For our purpose it is sufficient to examine accurately the growing point of Hippuris vulgaris. The construction is analogous in other cases. We cut off the terminal buds of very strong shoots to a length of about 1 cm., remove the leaves as well as possible, and prepare delicate longitudinal sections from the buds. In Fig. 120 is depicted the appearance of a very successful median longitudinal section. In order to render the arrangement of the cells of the vegetative cone clear and distinct, it is necessary to clear the sections. This is done by treating them with concentrated potash solution, and then after washing with water laying them in concentrated Acetic acid. The dividing walls of the cell layers, which are placed over one another like mantles, form a series of confocal parabolas. The outermost layer of cells, from which the epidermis proceeds, we term the dermatogen, *d*. Then follows the periblem, *pr*, made up of several layers, and yielding the cortex of the stem. Finally comes the plerome, *pl*, from which, as can be made out further down in the section, is derived the axile vascular bundle cylinder of the stem. In Hippuris, therefore, but not by any means in

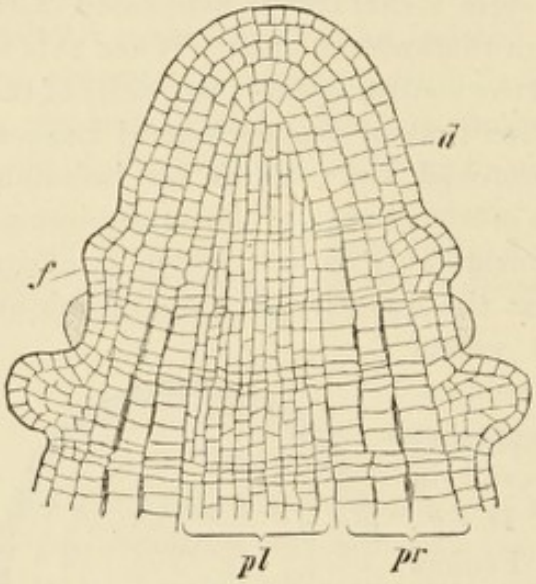

FIG. 120.—Longitudinal section through the vegetative cone of Hippuris vulgaris. *d*, dermatogen; *pr*, periblem; *pl*, plerome; *f*, leaf primordium. Magn. 240. (After Strasburger.)

all higher plants, there is a sharp division at the vegetative cone between dermatogen, periblem, and plerome.

The arrangement of the cells in growing points corresponds, according to Sachs,[1] with his principle of rectangular division. The anticlinals, *i.e.* the cell-walls which run at right angles to the surface of the growing point, and the periclinals, *i.e.* the walls curved in the same sense as the surface, cut one another at right angles. The anticlinal walls form, therefore, a series of orthogonal trajectories with respect to the periclinals. The fact that the cell-walls meet one another at right angles is to be observed very generally in the vegetable kingdom. We may observe rectangular division in its simplest form in filamentous algæ (*e.g.* Spirogyra).

Errera[2] and Berthold[3] have recently asserted that there is a still more general principle than that of rectangular division for the arrangement of the membranes. This is the principle of smallest surfaces. Many phenomena, *e.g.* this, that the cell-walls often meet one another, not at right angles, but at an acute angle (see Berthold, pp. 231 and 253), are only intelligible on the basis of this principle, as the above authors also show in detail. They arrive at the result that the grouping of the membranes is governed by the same laws as regulate the development of fluid sheets. This, as was shown particularly by Plateau, is determined by the principle of smallest surfaces, and it is therefore of interest to make some experiments on the subject.

We dissolve 3·75 gr. of powdered medicinal soap (to be obtained from the chemist) in a mixture of 187·5 gr. of distilled water, and 75 gr. of concentrated glycerin. The solution is once boiled, and may then be kept for a long time. We now make wire models of different bodies (cube, tetrahedron, cylinder), the wire being bent so as to form the edges of the structures. In the cylinder the upper circle is connected with the lower by three wires. Models about 50 mm. in height are quite sufficient. The soap solution is poured into a beaker, and the models are now dipped into it by means of a wire fixed on at a suitable place. On removing them soap sheets are seen stretching from edge to edge. These are arranged according to the principle of smallest surfaces, as is shown by special examination and mathematical calculation. If we destroy one of the sheets by carefully touching it with a glass rod, then a new state of equilibrium is set up in the system, often with production of very remarkable sheet forms, which however always satisfy the above principle.

The following experiment is very instructive:—A wire square of about 60 mm. side, resting on four wire pins, say 1 cm. high, is dipped in the soap solution by means of a wire fixed at a suitable place. The model, on removal from the solution, is placed in a horizontal position, and on the soap sheet is laid anyhow a thread, whose ends have been knotted together. If we pierce the sheet with a glass rod at a point within the area bounded by the thread, the sheet at once forms a minimal surface, and the thread consequently disposes itself in a circle.

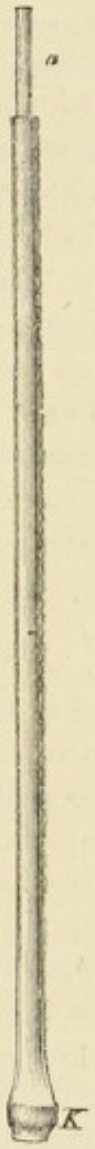

Fig. 121.—Lower part of an internode from the haulm of Secale. The part *a* has been raised by intercalary growth.

To examine the growing points of roots, we prepare median longitudinal sections from the roots of Zea Mais or Hordeum. Here also dermatogen, periblem and plerome are present; the root-cap, which covers the tip of the root, is very striking.

The cells of the growing points of stem and root are seen to be in a state of active division. The cells do not undergo vigorous surface growth, leading to elongation of the organs, until they are somewhat older.[4]

Regarding the further development of the elements formed in the punctum vegetationis, it is to be emphasized that this may take place either at the summit or at the base of the newly formed organ. We will here confine ourselves to what may be observed in the growth of the shoot axes of higher plants.

In grasses—but also in many other plants—the basal tissue of the internodes, surrounded by the leaf sheaths, retains for a considerable time a youthful character, while the upper parts of the internodes have already passed over into the state of permanent tissue. This remarkable fact of the existence of a basal intercalary zone of growth can be readily demonstrated by the following experiment:—We cut an internode from a haulm of Secale, and divide it into an upper and a lower half. We now place the two pieces with their lower ends in water, and

cover them with a bell-glass. At the end of twenty-four hours we find that, whereas no growth is to be observed in the upper half, growth has taken place in the lower half. In Fig. 121 is depicted the lower half of an internode of Secale after it has been kept for twenty-four hours in moist air. *K* is the node. The part *a* has been thrust by growth above the outer parts.

In the bean (Phaseolus), and in many other plants, the region of the internodes which is in a state of elongation, is situated at their summit. If at the upper end of the second internode of a bean, *i.e.* the segment of the stem which follows the epicotyl, we make two marks with Indian ink some distance from one another, while the third internode is already in a state of active elongation, it will be found at the end of twenty-four hours that the distance between the marks has considerably increased. The bean internodes are still growing at their upper ends after their lower ends have ceased to grow. Their behaviour is thus quite different from that of the internodes of grasses.

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[1] See Sachs, *Arbeiten des botan. Instituts in Würzburg*, Bd. 2.

[2] See Errera, *Ber. d. Deutschen botan. Gesellschaft*, 1886, p. 441.

[3] Berthold, *Studien über Protoplasmamechanik*, 1886, p. 219.

[4] It may be maintained, from the results of the recent researches of Zimmermann, Correns, Zacharias, Klebs, and others, that under normal conditions the surface growth of the cell membranes is brought about by apposition and intussusception. The former leads to the on-laying of fresh layers of cell material, the latter to the in-laying of fresh molecules of cellulose. The growth of non-turgescent cells (see *Untersuchungen aus d. botan. Institut zu Tübingen*, Bd. 2, p. 561) is effected by apposition alone; the normal growth in thickness of cell membranes (and also the growth of starch grains) likewise takes place by apposition.

152. Growth in Thickness.

Growth in thickness proceeds by no means in one and the same way in different plants and organs. We will here confine ourselves to the growth in thickness of the stem structures and roots of some dicotyledons.

We take first for examination a shoot axis of Aristolochia Sipho, 3–4 mm. in thickness, employing either fresh material, or alcohol material. In Fig. 122 is depicted the appearance of a delicate transverse section under slight magnification. We easily make out the general arrangement, and it may here be particularly

noticed that the vascular bundles, arranged in a circle, are still separated pretty considerably from one another. The fascicular cambium, *fc*, of the separate bundles, composed of small radially arranged cells, is continued between them to form the interfascicular cambium, *ifc*, developed from the parenchyma of the ground tissue, and thus a closed cambium ring is produced.

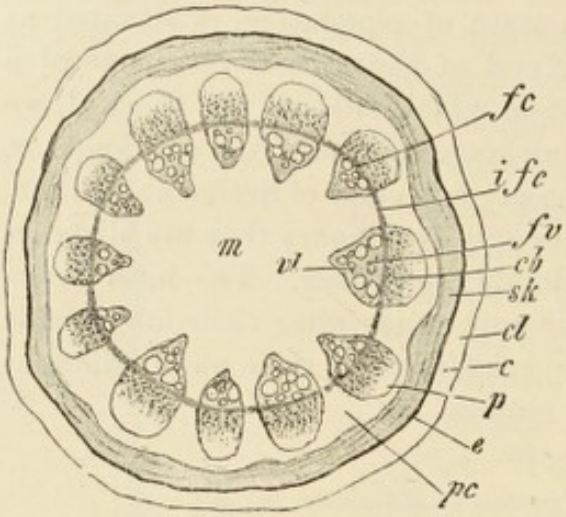

FIG. 122.—Transverse section of a twig of Aristolochia Sipho, 5 mm. in thickness. *m*, pith; *fc*, vascular bundles; *vl*, their vascular region; *cb*, sieve region; *ifc*, interfascicular cambium; *p*, phloem parenchyma outside the sieve region, affording transition to the ground tissue; *pc*, pericycle; *sk*, ring of sclerenchyma; *e*, starch sheath; *c*, green cortex; *cl*, collenchyma. Magn. 9. (After Strasburger.)

As the shoot continues to develop, the cambium (here only the fascicular cambium) produces on the inside secondary wood, on the outside bast tissue. The growth in thickness thus brought about results chiefly in a very considerable increase in the wood of the fibro-vascular bundles, as we at once discern if we examine transverse sections from Aristolochia stems 10 mm. in thickness. We also perceive in these perhaps ten primary medullary rays, which traverse the wood in its entire thickness, and therefore extend from the cambium to the pith. Secondary medullary rays are present in considerable numbers. It is further to be noted that at the periphery of the older shoots a formation of periderm has set in, and that the closed ring of sclerenchyma present in the younger parts (see Fig. 122, *sk*) is broken up into separate pieces.

The hypocotyl of Ricinus communis also affords a favourable object for the study of the changes which take place in stems during growth in thickness. In Fig. 112, p. 309, is depicted the appearance of a transverse section of a vascular bundle of a fully elongated hypocotyl of Ricinus. We use alcohol material, and make out without any trouble the presence of the fascicular and interfascicular cambium. During growth in thickness, secondary wood is deposited on the inside and secondary bast tissue on the outside.

We prepare a section from the upper part of a root of a seedling

of Phaseolus multiflorus which is just beginning to form the first secondary roots. We perceive the epidermis, the cortex, and the central cylinder enclosing the vascular bundles. This is surrounded in its entire circumference by a characteristic tissue which we term the endodermis. It is specially characteristic of roots that the wood and bast of their vascular bundles are arranged in a manner essentially different from that in shoots. There are, viz., in roots several xylem bundles, with which alternate as many phloem bundles situated nearer to the periphery of the central cylinder. A transverse section of a bean root shows us four xylem and four phloem bundles. We consequently term it a tetrarch root. As with advancing development the root increases in thickness, the tissue between the xylem and phloem bundles is converted into cambium. A closed cambium ring originates, which yields on the inside secondary wood, on the outside secondary bast

153. Apparatus for Measuring Growth Movements.

For demonstrating growth movements, an arrangement first employed by Sachs, and known as the arc indicator, is very serviceable. This piece of apparatus, represented in Fig. 123, in the form constructed by Pfeffer, may be obtained of Albrecht in Tübingen at a price of 60 mks.

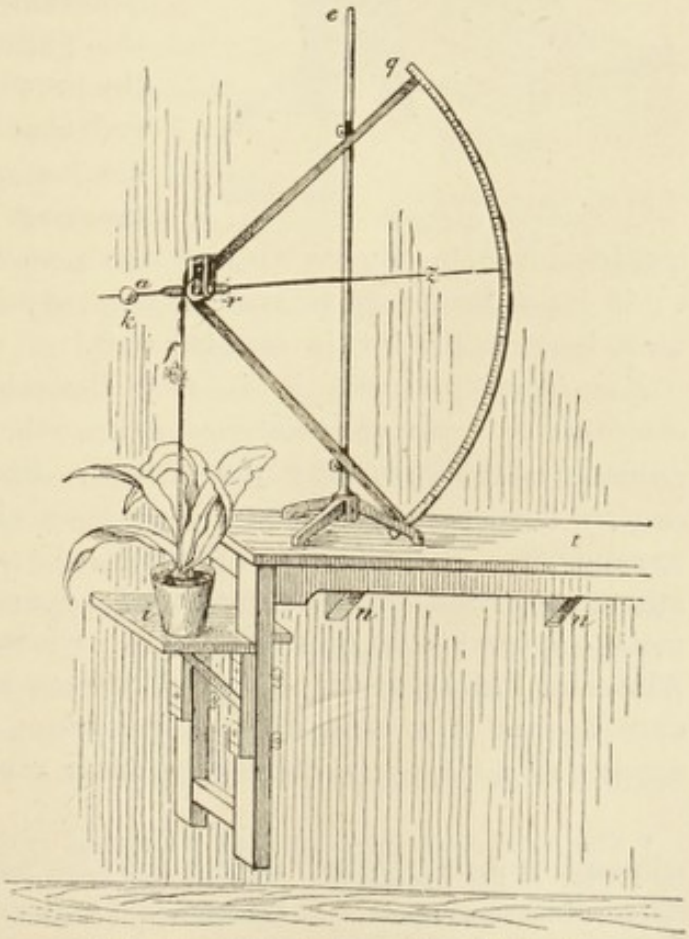

Fig. 123.—Arc indicator. (After Pfeffer.)

The thread, *f*, connected with the plant, is carried over the pulley, *r*, which is fixed at the centre of the quadrant, *q*. I

chiefly used for investigation seedlings of Phaseolus grown in flower-pots, in good garden earth, and with the epicotyl projecting about 2 cm. from the soil. The thread can easily be fixed to the plant by a loop. The pulley bears on one side the indicator, *z*, and on the other the arm, *a*, which is pierced to permit the passage of the thread, *f*, and carries a movable weight, *k*, which serves to balance the weight of the indicator, or give a certain overweight on one side. It is best to balance the indicator exactly, and obtain the desired excess weight by a thread suitably carried over the pulley and stretched by a weight. The quadrant has a radius of 70 cm.; the pulley, *r*, is small, so that the growth movement is magnified about 43 times by the indicator, *z*. The indicator is a tapering brass tube. The quadrant can be moved up and down on the heavy iron stand, *e*; it must be set up in such a way as to be vibration-free, *e.g.* on a bracket fixed to a massive wall.

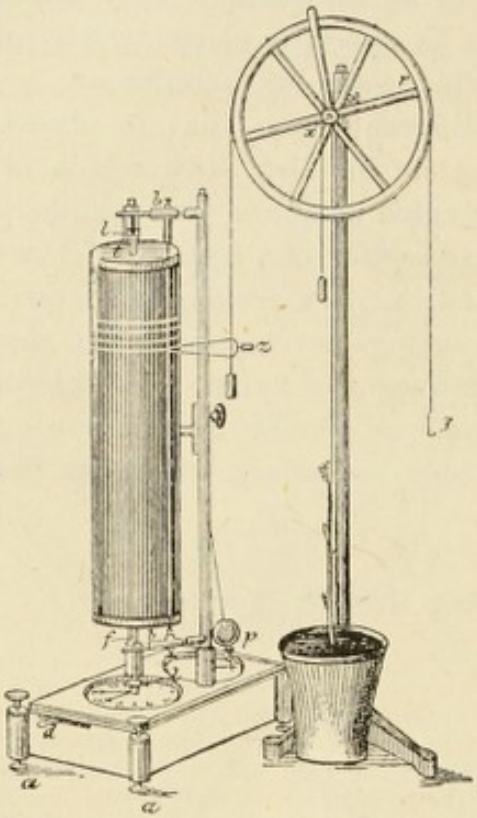

Fig. 121.—Auxanometer. (After Pfeffer.)

For many purposes it is very desirable, and indeed often essential, in accurate researches on growth, to have apparatus for automatically registering the growth. Sachs[1] was the first to construct an instrument for the purpose, viz. the self-registering auxanometer. After him Wiesner, Baranetzky, Pfeffer,[2] and others described modified forms of apparatus, and perhaps the most serviceable is that of Pfeffer, which may be obtained of Albrecht, Tübingen, at a price of 320 marks. For success in the experiments it is naturally an important condition to have the apparatus set upon a table free from vibrations.* The thread

* The instrument here described and figured is the same as that mentioned by Pfeffer in his handbook. Recently the auxanometer has been still further improved in some respects by Pfeffer, and the newer form may be obtained at the same price from Tübingen.

attached to the plant is taken over the small wheel, *x*, which is cemented on the large wheel, *r*, and accurately centred about the same axis. A thread fixed to the larger wheel, and wound round it, carries the indicator, *z*, which in our arrangement sinks as growth proceeds. The indicator is balanced by a weight, *g*, fastened to a thread wound in the opposite direction. The drum, *t*, coated with soot, is driven by clockwork, actuated by a spring, and regulated by a conical pendulum, *p*. The clockwork is contained in a heavy iron case. The drum, 70 cm. long, which may be fixed centrally or excentrically by displacement of the supporting axis, *f*, may moreover with the axis be entirely removed (at *l*). It makes one revolution per hour. The indicator, which must not be too light, is made of brass, and horizontal; it is arranged, as the figure shows, to trail on the drum, against which it is made to press by giving the thread a twist. If the drum is fixed excentrically, the indicator only strikes it at intervals, and between times slides on the catgut stretched between the movable clips, *b*. In the arrangement here described, the auxanometer magnifies the growth fifteen times.

The thread (silk thread) can easily be attached to the plant by making a loop at one end of it, passing the other end through it, and finally laying the slip-loop thus made over the upper end of an internode, immediately below the base of a leaf.

In order to limit as far as possible sources of error arising from hygroscopic peculiarities of the thread, it is advisable to employ silk thread only for the part passing over the pulley, and connecting with the plant, for the rest using fine silver or platinum wire, sharply bent at both ends, so that small loops of the thread can be hooked on.

If the observations are made in the light, a mirror must be placed in a vertical position behind the plant, and parallel with the window, in order to exclude disturbing heliotropic curvatures. The experiments, moreover, are to be carried out in a place subject to the slightest possible variations of temperature. Temperature readings must of course always be made (for details see 77). The soil, in which the plants must have been rooted for a long time, is well watered some time before the investigation, and must not get dry during the observations.

Before being used, the rotating cylinder of the apparatus has to be coated with paper. To do this we lay on the table a sufficiently large piece of paper, glazed on one side, go over the rough side of

the paper uniformly with a moderately moist sponge, gum both long edges, and roll the cylinder over the paper. When the paper has become dry, we pass the cylinder to and fro over a large, broad turpentine flame so as to coat it uniformly with soot. When the auxanometer is in motion with the drum placed excentrically, the indicator, *z*, set in movement by the growth of the plant, comes in contact for a time every hour with the rotating cylinder, and removes the soot from its surface at the places of contact. The lines thus produced are fixed by passing the paper after removal from the drum through an alcoholic solution of colophonium, and drying. If we measure the distance between the lines, we obtain a direct measure of the growth movement. Sachs has so fully described the whole method of procedure, and the sources of error which it presents, that we must refer for details to his cited work, especially pp. 116, 118, and 119.

The self-registering auxanometer may be used for many researches on growth. It is especially indispensable when it is desired to obtain exact information as to the character of the daily period of growth of internodes (see further below).

For accurate measurements of very small growth movements of vertically growing structures, a horizontal microscope (or telescope) is essential. The Quincke-Pfeffer apparatus represented in Fig. 125 is very suitable. It may be obtained from Albrecht, in Tübingen, at a price of 110 marks. The tube of the microscope is focussed on the object under investigation by means of the screw, *t*. The pillar, *s*, moving in the tube *h*, serves for coarse adjustment in the vertical direction, while the millimetre screw, *m*, serves for fine adjustment, and for re-focussing when the image of the object has run through the scale of the ocular micrometer. By means of this accurately cut screw we can at once, as with a cathetometer, measure the distance between marks not simultaneously included in the field of view. For example, one revolution of the milled head may correspond with 0·792 mm., and $\frac{1}{2}$ divisions can be accurately read on the scale below it, which is

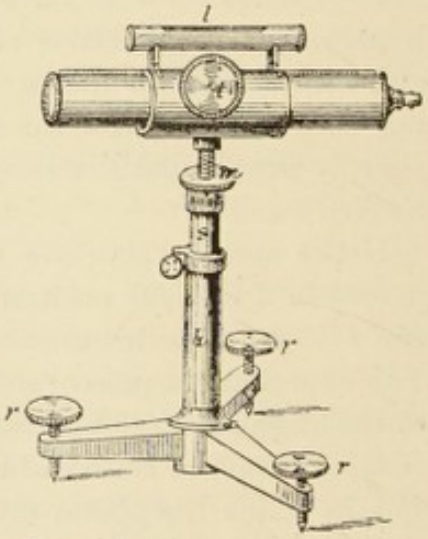

Fig. 125.—Horizontal measuring microscope. (After Pfeffer.)

divided into 100 parts. The microscope is levelled by means of the levelling screws, *r*, and the spirit level, *l*.

It remains to be remarked that the focal distance of the microscope for 20-fold magnification is 80 mm. The ocular micrometer is divided into 120 parts. Each division, with 20-fold magnification, has a value of 0·07 mm.; one revolution of the screw as we have said corresponds with 0·792 mm. The screw permits the measurement of distances up to 3 cm., the micrometer distances up to 8 mm. Both ways of measuring may be employed.

In using the microscope here described, it is exceedingly important that it should be fixed so as not to vibrate, *e.g.* on a suitable bracket, or on a substantial table, supported by a strong wooden tripod, and movable in a vertical direction. The support also carries a clinostat (see Section V.), whose axis is vertical, so that its disc rotates slowly in a horizontal plane (say once every hour). As research material we may first work with sporophores of Phycomyces or Mucor.

A piece of bread is moderately moistened with 0·1 per cent. grape-sugar solution, then sterilised by exposure for some time to a temperature of 100° C. in the drying chamber, and finally sown by means of a sterilised needle with a few spores of Phycomyces or Mucor.* Mucor spores may readily be obtained as described in 36. The bread is left under a bell-glass, in the dark, till the sporophores have attained a suitable size; it is then placed in a glass dish on the disc of the clinostat, and again covered with a bell-glass, which, however, must be tubulated for the accommodation of a thermometer. The sporophores now grow straight upwards, even in the light, when the clinostat is set in motion. Under the conditions indicated, heliotropic curvatures cannot take place. Before starting the clockwork of the clinostat, we focus the microscope on a sporophore in such a way that the top of the sporangium appears to touch one of the divisions of the ocular-micrometer. If we now start the experiment, the disc of the clinostat making one complete rotation per hour, it will be found that the sporangium is not again distinctly in the field of view for an hour. The amount of growth made during the hour may readily be determined by rotating the screw, *m* (Fig. 125). Not infrequently our sporophores grow 1–2

* If a few sporangia are transferred to sterilised water, they burst and liberate their spores. It is best to infect the bread with a few drops of the spore-containing fluid so obtained.

mm. per hour, and the experiments may be continued for a long time (for further experiments after this method see below).

If it is desired to determine the growth in short intervals of time of the stems of seedlings (*e.g.* in the hypocotyl of Lepidium), fine ink lines must be painted on them with Indian ink. Soon after their development has begun, the seedlings are fixed in small glasses by means of cotton wool, in such a way that their roots dip into water, or they may be cultivated in small clay cylinders containing sawdust. They are placed under a bell-glass and subjected to slow rotation on the clinostat. Sharp angles or projections of the ink-marks serve as points of reference for the measurements.

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[1] See Sachs, *Arbeiten d. bot. Instit. in Würzburg*, Bd. 1, p. 113.
[2] Pfeffer, *Handbuch d. Pflanzenphysiologie*, Bd. 1, p. 86.

154. The Grand Period of Growth.

It is a fact of fundamental physiological significance that all growing plant structures (roots, stems, leaves, etc.), even under constant external conditions, do not experience the same amounts of growth in equal successive intervals of time. Every part at the commencement of its development grows slowly; gradually its rate of growth becomes more rapid, attains a maximum, then becomes more slow again, and finally growth completely ceases. To prove this in a general way first of all, it is sufficient to soak a few pea seeds, and lay them in a crystallising glass containing enough water to half cover them. Or by means of cotton wool we fix a pea or bean seed which has just germinated in the hole of a cork which closes a glass vessel containing water, so that the root of the seedling grows downwards in the water. Germination is allowed to proceed in the dark, at as constant a temperature as possible (say 20° C.), and we ascertain every day at a particular hour the length of the roots. It is found that the growth of each root is at first comparatively slight, gradually becomes more considerable, sooner or later (in my experiments, made at a temperature of 16° C., on the ninth day) attains a maximum, and then gradually falls off again.

We soak good seeds of Pisum, Phaseolus, or Vicia Faba, for twenty-four hours in water. The seeds are then put into moist sawdust, which has previously been rubbed down between the flat hands, and filled into large wooden boxes or flower pots to form a loose seed

bed. Care must be taken that the emerging roots will not have to make curvatures in order to grow vertically downwards. The Vicia seeds we lay in the sawdust with the micropyle directed downwards. The Phaseolus seeds are laid horizontally, so that the emerging main root forms a right angle with the long axis of the seed. When the roots have attained a length of 1·5–2 cm., the seedlings are removed from the bed, carefully washed, dried with a piece of soft linen, and provided with marks. We use the best black Indian ink, rub it with a little water on a porcelain plate, and paint fine lines on the roots with a sable pencil. The distance between the marks may be 1, 1·5, or 2 mm., according to circumstances. The first line is therefore 1, 1·5, or 2 mm. distant from the growing point,* the second 1, 1·5, or 2 mm. from the first, and so forth. It is best to take distances of 1 mm. In marking the roots it is convenient to proceed as follows:—We take a sheet of cork, say 2 cm. thick, along the left edge of which a number of large notches have been made with a round file; from each of these, along the surface of the cork, run in different directions a few grooves made with thin round files. We now find a notch in which the seed with a little coaxing will stick, its root at the same time lying in one of the grooves. Alongside the root we lay a millimetre scale, in such a way that we can draw the lines on it as continuations of the divisions of the scale. The seedlings, whose roots have been marked, are now fixed in glass cylinders by means of long pins in the manner indicated in Fig. 126. I used cylinders about 30 cm. in height, and 7–8 cm. in diameter, and also much larger ones, which are preferable. The cork, *K*, into which the pins are stuck, is cemented on the bottom of the stopper with sealing-wax, and is soaked with water. The bottom of each cylinder is covered with a layer of water, so that the roots are surrounded by moist air. If it appears to be necessary, we may further sprinkle

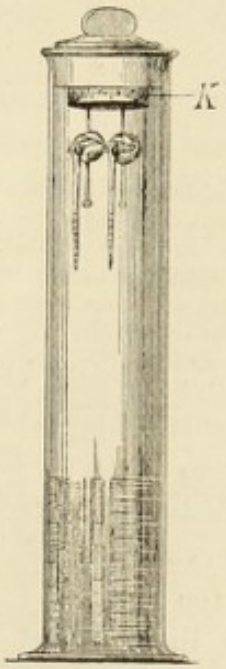

Fig. 126.—Glass cylinder for culture of seedlings.

* The growing point is of course only seen indistinctly glimmering through; it is situated about 0·2–0·5 mm. from the root tip.

the roots now and then with a little water, or line the cylinder with moist blotting-paper. We place the cylinder in the dark, and expose the seedlings to a temperature as constant as possible (*e.g.* 20° C.). We may also allow the roots in the cylinders to grow in water. We use vessels of about 3 litres capacity, half filled with water, fix the seedlings on very long pins, and let the roots, but not the receptacles of reserve material, dip into the water.

After twelve or twenty-four hours it can readily be determined by measuring that the growth in the youngest partial zone, next to the growing point, has not been excessive. In the next zone more energetic growth is exhibited. It is one of the following zones in which most rapid growth has proceeded. Then come zones in which it is found that the growth has been slower again, and the oldest parts of the roots have undergone no growth at all (see Fig. 127). The older partial zones have already passed beyond the stage of strongest growth; the youngest have not entered upon it. Each transverse zone of a structure, like that organ as a whole, at first grows slowly, then more rapidly, attains a maximum rate of growth, and finally grows more slowly again. Hence we find also that the youngest transverse zones of a root, which *e.g.* during the first twelve hours have not grown very much, during the following twelve hours already grow more actively. At a particular time naturally the maximum of growth lies in these zones, but later still the rapidity of growth diminishes again. It is instructive to observe the growth of the partial zones of a root for a considerable period, determining the increments of growth at intervals (which must, however, be comparatively short, say six to ten hours). A difficulty, which, however, is not insuperable, lies in the fact that the ink-lines placed on the root become disintegrated.*

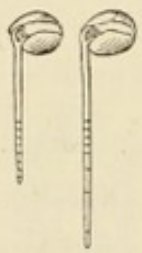

FIG. 127.—On the left a pea seedling on whose root ink-lines have been painted. On the right the same seedling after the root has been growing for some time.

In investigating the growth of the root, the student will not escape the important fact that the length of the growing region, *i.e.* the length of that portion of the originally marked region

* See Sachs, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 421. To avoid mistakes it is better each time to make a fine ink-line in the middle of the marks, and use this line as the point of reference for the further measurements. Consult Sachs also for further particulars respecting the methods here indicated.

in which, after a certain time (say twenty hours), any growth is found to have taken place, is always very small. According to the kind of seed, viz., and the individuality of the separate objects, the growing region of the root (*e.g.* in Pisum, Phaseolus, etc.) is only about 4–8 mm. in length. The growing region of the stem, on the contrary, is, as we shall see, much longer.[1]

We now proceed to follow closely the grand period of growth in the stem, and there is no special difficulty in determining the general course of the growth in this organ. We grow seedlings of Phaseolus or Pisum, rooted in loose garden earth, in absence of light, keeping the temperature as constant as possible. By single measurements made from day to day it can be proved that the internodes (the epicotyl or succeeding internodes) at first grow slowly, then more rapidly, at a particular time exhibit a maximum rate of growth, and then again grow more slowly. Seedlings growing in darkness (I experimented, for example, with Pisum plants, produced from large seeds) may, under some circumstances, produce very long stems, consisting of quite a large number of internodes. One plant which I had under examination produced a stem more than 500 mm. in length, and composed of seven internodes. If we measure the length of the mature internodes we find that the oldest are comparatively short, then come longer ones (in my experiments the fifth was the longest), and the youngest internodes are then again shorter. It is very generally observable that the successive internodes, when fully elongated, are not of the same length, a fact which finds a simple expression when we say that the growth energy of the different segments of the stem varies, owing to internal causes.

We grow seedlings of Phaseolus multiflorus in the dark in flower pots. When the epicotyls have attained a length of about 50 mm., we make, on the most strongly developed stems, a number of ink-lines at intervals of 3 to 5 mm. (for method see 147 and 148). The seedlings are then again placed in the dark, the temperature being kept as constant as possible. Every twenty-four hours we determine by measurement the amount of growth in the different partial zones. The growing region of the stem, in contrast with that of the root, is very extensive. I found, for example, that a length of 35 mm. of the Phaseolus epicotyl was in a state of growth. In the youngest (uppermost) partial zone, the growth at the beginning of the

investigation is not very considerable. In the next it is already more active. The maximum growth takes place in the third or fourth zone; in the following ones the rate of growth again diminishes. If we continue the observations for some time, it will be found that the growth soon ceases in the older zones, while the maximum of growth is no longer situated in the third or fourth, but in a younger zone. Still later the rate of growth in these latter partial zones in turn diminishes.[2]

In order to prove the existence of the grand period in the growth of leaves, we grow cucumber or tobacco plants in large flower pots, and, when a few leaves have unfolded, place over them large bell-glasses, keeping them in a place where the temperature is as constant as may be, *e.g.* in a room with a north aspect. The research material is left exposed to the light. Near the base of the blade of some of the young leaves we place a dot of ink to serve as a mark. Every day we measure with a millimetre scale the distance between the tip of the leaf and the dot at its base. Naturally the temperature is always to be carefully noted. During the months of May and June I followed carefully the growth in length of leaves of Aristolochia Sipho growing in the open air, and found at first, when the temperature was fairly constant, that the phenomenon of the grand period of growth was very clearly exhibited. The growth during the twenty-four hours was at first only 5 mm., then 7 mm., later on 10 mm. Later still, owing to considerable variations in the temperature, great irregularities became observable in the growth of the leaves, but it is nevertheless instructive to repeat such observations, since they show us how important it is, in studying the grand period of growth in plant structures, not to leave out of consideration, for a moment, external influences affecting their growth.[3]

In order to determine the causes of the grand periods of entire organs, we must, as I have emphasised in my *Lehrbuch der Pflanzenphysiologie*, p. 249, explain the variations exhibited during development in the rate of growth of the separate partial zones of the structure. This is done, as far as at present appears possible, in 147, so that we must here refer the student to the account there given. At the commencement of growth in an entire organ, we have at first only the sum of few and insignificant increments of growth, later more and larger ones, till finally the increments of growth again become inconsiderable.

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[1] See Sachs, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 413.
[2] *Ditto*, Bd. 1, p. 99, and Wortmann, *Botan. Zeitung*, 1882.
[3] See Prantl, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 371.

155. Rate and Energy of Growth.

Daily experience teaches us that the rate of growth of plants varies very considerably. Even the separate individuals of one and the same species under similar external conditions, exhibit different rates of growth. The experimenter has always to take account in his researches of individual differences of behaviour in the objects under investigation, such differences often influencing the success of the observations in a very unpleasant manner. It is therefore instructive to make the following experiment. Peas, beans, or other seeds, as normal and uniform as possible, are germinated in large numbers in damp sawdust. After some time, accurate measurements of roots, stems, and leaves are made, and from these we learn that corresponding organs of different individuals of the same species, in spite of their having all developed under precisely the same external conditions, have by no means grown at the same rate. The individual differences of behaviour in the separate plants, which are often considerable, are clearly brought out in such observations.

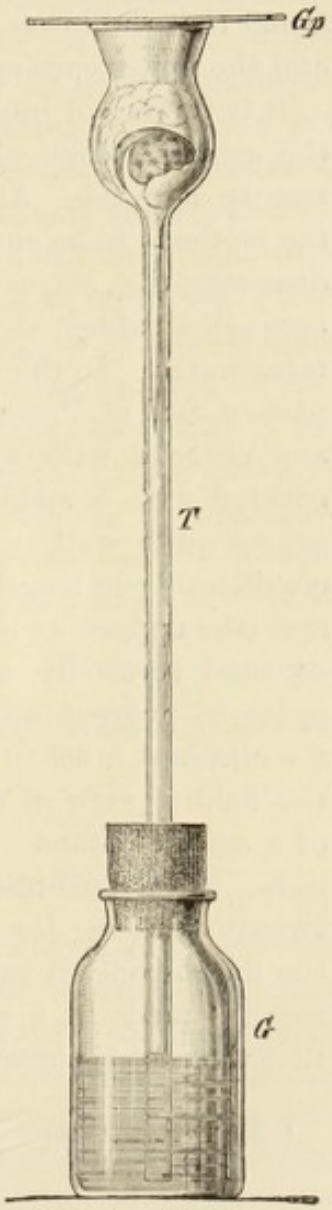

FIG. 128.—Apparatus for investigating the growth of roots.

Under similar external conditions, however, homologous organs of different species of plants also exhibit specific differences in their rate of gowth. The stems and leaves of Aristolochia Sipho and Humulus lupulus, for example, grow comparatively rapidly; the corresponding organs of other plants very slowly. Again, the stems, *e.g.* in Polygonum Sieboldi, grow very rapidly. I found, *e.g.*, that a shoot of this plant, which on May 3rd was 60 cm. high, had, after twenty-four hours in warm, damp

weather (evening temperature at 11 o'clock still 15° C.), reached a height of 71 cm.

The energy of growth is a function of the duration of the growth and the rate of growth. The energy of growth of the individual internodes of a stem, and therefore their ultimate size, is not the same. If, for example, pea seedlings are grown for some weeks in darkness, and we determine the length of the different internodes when the stem has quite ceased to grow, it is found that the lower internodes are short, the middle ones long, and the upper ones again shorter.[1]

It is further of interest to learn that it is possible to determine the growth which plant structures undergo in a short time, say twenty minutes. Attention has already been drawn, in 153, to the methods to be employed in accurate observations. Here is a demonstration experiment. A thistle tube (*T*, in Fig. 128) passes through a rubber stopper closing the glass bottle *G*, which contains water. In the upper, expanded part of this thistle tube we place a seed of Pisum or Phaseolus, germinated in moist sawdust, and cover it with wet cotton-wool. The mouth of the tube is covered with a small glass plate, *Gp*. The root of the seedling grows quite well in the moist air surrounding it; it increases considerably in length. We now place our apparatus on a clinostat (the apparatus is described and figured below), whose axis is directed vertically, and put the seedling into slow rotation, in order to prevent any heliotropic curvature of its root. Before the clinostat is set in movement, we bring the tip of the root into the field of view of a microscope, directed horizontally by means of a suitable stand. We naturally employ only slight magnification. Now the clinostat is set rotating, and when, say after twenty minutes, the root again appears in the field of vision of the microscope, it can be demonstrated, especially if the experiment is made at a comparatively high temperature (20° to 25°) that the root has grown.

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[1] See Detmer, *Lehrbuch d. Pflanzenphysiologie*, 1883, p. 248.

156. Torsions.

Torsions are frequently to be observed in internodes, and also in leaves. Beautiful examples of torsion are exhibited by the older internodes of twining stems, to which we shall return elsewhere, and in plants grown in darkness. Thus, for example,

flower stalks of Hyacinthus orientalis, grown in absence of light, are frequently much twisted; and so also is the hypocotyl of Helianthus annuus under similar circumstances, while the corresponding organ of seedlings grown under normal conditions exhibit no torsions. If seeds of Helianthus annuus are laid in damp sawdust and cultivated, some in the dark, and some in the light, we can easily satisfy ourselves that this is really the case. It is seen also that the torsions in the etiolated hypocotyls do not appear till towards the end of their growth in length. In many cases torsions are due to internal causes. Others, to which attention may be drawn at once, originate in quite a different manner. The stem of a vigorous marrow plant grown in a pot is tied to a stick in such a way that it cannot execute curvatures. On the upper side of some of the leaf-stalks, and along the midribs of their laminæ, we make a line of ink-dots; and then, after the soil in the pot has been secured by strips of wood, the plant is inverted and placed in the dark. Owing to various causes (geotropism, photoepinastic after-effects) the leaf-stalks, in the course of a few hours, bend upwards; but since the weight of the laminæ borne by the stalks is hardly ever equally distributed on both sides of the plane of curvature, torsions are produced, of whose extent we can easily judge by observing the ink-marks, which no longer lie in a straight line. These torsions may be rendered permanent by growth.[1]

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[1] See H. de Vries, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 268; and Sachs, *Lehrbuch d. Botanik*, 1874, p. 833.

157. Some Examples of Spontaneous Nutations.

We lay a few soaked seeds of Vicia Faba in loose, damp sawdust, micropyle downwards. If we examine our seedlings carefully just when the stem begins to emerge between the cotyledons, we shall find that they have straight roots, directed vertically downwards. We now fix a number of Vicia seedlings in a suitable receiver, and exclude the light. At the end of twenty-four hours we find that the roots have departed from their original vertical direction. The roots are curved in the manner represented in Fig. 129, a result due really to a curvature in the hypocotyl and upper parts of the root. The advancing root tip, in consequence of the nutation which has taken place, naturally comes to lie

obliquely to the vertical, and therefore seeks, in virtue of its geotropic irritability, to turn downwards in a curve. Seedlings of various other Papilionaceæ behave in these respects like the seedlings of Vicia; and it is further to be observed that the roots exhibit the nutation referred to, not only in a moist atmosphere, but also, although not to the same extent, when grown in loose earth or sawdust. If in the seedlings of the Papilionaceæ we consider the posterior side to be that on which the convexity of the stem lies (see Fig. 129, *H*), the anterior, *V*, that towards which our roots always turn, then the median plane of the seedling corre-

Fig. 129.—Seedling of Vicia Faba.

Fig. 130.—Seedling of Phaseolus multiflorus, upper portion.

sponds exactly with that in which the two cotyledons are in contact with each other. The fact that the curvature of the root, resulting from the nutation of the hypocotyl and base of the root, always takes place in the median plane of the seedling, must, for obvious reasons, be carefully noted in studying the behaviour of roots placed in a horizontal position. Thus, *e.g.*, Vicia seedlings on a horizontal surface must be placed so that they lie with their right or their left side—*i.e.* the outer face of one of their cotyledons—on this surface.[1]

Interesting examples of nutation are also to be observed in the first segments of the stem in many dicotyledons; and we will study these with some exactness, selecting for observation Phaseolus multiflorus. If we split a seed which has been placed

in water till thoroughly soaked, we observe a somewhat considerable curvature at the apex of the stem of the embryo which lies between the cotyledons. During the germination of the seed this curvature becomes still more marked, so that the terminal bud emerges from the soil completely nodding over. The convexity of the curvature now, and also later, lies on the side of the epicotyl remote from the cotyledons—*i.e.* on the posterior side of the epicotyl—and the nutation takes place in consequence of more rapid growth of this posterior side (see Fig. 130). At *a* lies the convexity of the curvature. If we grow our Phaseolus plants constantly in darkness, the nutation at the upper end of the seedling stem persists for a long time; it is only in the last stages of germination that the terminal bud directs itself vertically upwards. This takes place very quickly, on the contrary, when young seedlings—*e.g.* seedlings whose terminal bud has just broken through the soil—are exposed to the influence of bright, diffused daylight. The curved epicotyl then speedily and completely straightens.

If we make an ink-mark on a nutating epicotyl of Phaseolus at the place of greatest curvature (Fig. 130 at *a*), we find this mark after twenty-four or forty-eight hours, the seedling having naturally been kept during that period in darkness, at *b*. From this it is clear that the originally nutating parts of the epicotyl gradually in the course of growth straighten. The nutation is transmitted to the newly forming parts of the stem. More exact consideration of the marks also frequently shows, however, that the plane of nutation is not always the same. The nutation must be regarded as quite spontaneous. It takes place even when the seedlings are slowly rotated in the dark round a horizontal axis. For further information see the section on experiments with the clinostat.

The angle formed by the nutating part of the epicotyl of Phaseolus usually amounts to 180°, so that the end bud of the upwardly growing stem is directed vertically downwards. In more accurate observations, however, especially of very actively growing Phaseolus seedlings, we find that the angle does not always remain the same. If one day it is 180°, it may on the following be, say, 90°; on the third 145°.[2]

It appears that most structures exhibit spontaneous nutations. To this class belong also the circumnutations of many seedlings;[3] and if we grow, *e.g.*, oat seedlings in the dark, we can

make out by repeated observations—*e.g.* every quarter of an hour—that the tip of the plumule, when about 1–2 cm. long, is carried round in space in a more or less circular line. I do not, however, propose to study circumnutation, since the whole subject stands in need of searching critical investigation, and especially more attention than hitherto must be paid to the influence of external conditions.

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[1] See Sachs, *Arbeiten d. bot. Inst. in Wurzburg*, Bd. 1, p. 402.
[2] See Wortmann, *Botan. Zeitung*, 1882, No. 52.
[3] See Darwin, *Movements of Plants.*

II. THE CONDITIONS NECESSARY FOR GROWTH AND THE INFLUENCE OF EXTERNAL CONDITIONS ON GROWTH MOVEMENT.

158. Growing Plant Structures Require Material for Growth.

The growth of a plant structure can only take place in a normal manner when the necessary material and energy for the growth of the individual cells are available. If, for example, seedlings grow in complete darkness, the growth of the parts ceases when the store of reserve material is exhausted. It is instructive to study in some detail the connection between the energy with which growth takes place and the stock of reserve material. For this purpose we sow seeds of Phaseolus multiflorus in garden earth in flower-pots. One pot is provided with as large seeds as possible; a second with similar seeds, from each of which, however, when germination has just begun, and the main-root has broken through the seed-coat, one of the two cotyledons has been removed. In a third flower-pot we sow small Phaseolus seeds. Germination may take place in darkness or in the light. We find that the plants derived from large seeds develop more vigorously than those from small seeds, or from those deprived of one of their cotyledons. In my investigations, which were conducted in the light, there were at first no very striking differences in the appearance of the seedlings. Such differences did not appear till the first trifoliate leaf had unfolded, and the third internode was actively elongating. The plants derived from large seeds had considerably larger leaves and longer internodes than those from

small seeds, or from seeds robbed of one of their cotyledons. The measurements of the different parts of the plant are easily made. The stock of reserve material in bean-seeds is very large, so that the first stages of germination in my experiments could, in all cases, proceed normally. At a later stage, although assimilatory activity was not excluded, a considerable difference in the growth of the plants became apparent, and this could only be referred in the main to the more or less considerable stock of reserve material in the cotyledons of the seeds.

159. The Quantity of Water in Plants and their Growth.

Normal growth of the cells is only possible when they contain an adequate quantity of water. This fact is easy to understand from various considerations. Here it need only be pointed out that active growth presupposes energetic turgor-extension of the cells, which again is only possible when the tissues are rich in water. If the turgescence of the cells sinks, owing to loss of water, their rate of growth also at once diminishes. We germinate maize, pea, or bean seeds in sawdust. When the roots have attained a length of a few cm., we make fine ink-marks on them at a distance of 2 cm. from their tips, and fix the seedlings in suitable glass vessels in the manner indicated in 154. These vessels we fill with different fluids; one with spring water, a second with a 0·5 p.c. solution of Potassium nitrate, a third with a 1·0 p.c. solution of the same salt, and a fourth with a 2·0 p.c. solution of it. The roots must reach vertically downwards into the fluids. At the end of twenty-four or forty-eight hours we determine the amount of growth which the roots have experienced. In each case we employ three or four seedlings, and take the average. It is found that the roots in contact with spring water grow the most actively. With increasing concentration of the Potassium nitrate solution, however, their growth becomes less vigorous, from the fact that the solutions are now able to remove water from the cells, and consequently reduce their turgescence. In fairly concentrated solutions of saltpetre (*e.g.* 10 p.c. solutions) the roots do not grow at all. Indeed, they become shorter since they pass into a state of plasmolysis.[1]

It is remarkable that fungi continue to grow in fluids whose concentration is much too great for the growth of higher plants. We prepare a solution containing in every 100 parts of water, 0·4

parts of Ammonium nitrate, 0·2 parts of acid Potassium phosphate, 0·02 parts of Magnesium sulphate, and 0·01 parts of Calcium chloride. Quantities of 50 c.c. of the fluid are placed in small glass flasks, and treated respectively with 5 gr. (10 p.c.), 10 gr. (20 p.c.), 25 gr. (50 p.c.) of grape-sugar. The vessels, stopped with cotton-wool, are sterilised in the steam apparatus. For observation we select Penicillium glaucum. The fluids are infected with spores of this fungus, and then all exposed to the same external conditions. It is found that development of the fungus takes place even in the 50 p.c. solution, though certainly it grows much more slowly in this than in the others. The causes of the growth of the cells in fluids of high concentration is perhaps to be sought in the fact that these act as stimuli, and so affect the metabolism that an adequate elevation of the osmotic capacity of the cell contents is produced.[2]

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[1] See H. de Vries, *Untersuchungen über die mechanischen Ursachen der Zellstreckung*, Halle, 1877, p. 56.

[2] See Eschenhagen, *Einfluss von Lösungen auf das Wachsthum von Schimmelpilzen*, Stolp, 1888.

160. Respiration and Growth.

As has already been pointed out in 108, some plants can grow even in complete absence of free Oxygen. Most plants, however, are only capable of growth when free Oxygen stands at their disposal. This fact can easily be proved as follows.[1] Two retorts of about 90 c.c. capacity (see Fig. 11) are filled with distilled water, which has been boiled and then allowed to completely cool again without exposure to the air. In each retort we place some air-dry wheat grains or pea seeds, and then invert it with its mouth under mercury. After twenty-four hours, when the seeds have swollen, we replace the water of one retort almost entirely with atmospheric air, that of the other with pure Hydrogen. This we prepare by treating Zinc free from Arsenic with dilute Hydrochloric acid, and pass the gas through aqueous solutions of Potassium hydrate and Potassium permanganate, to free it from injurious substances. In contact with the air the seeds soon germinate; in Hydrogen, germination does not take place at all. If, however, the seeds have not been kept too long in the Hydrogen (say only two or three days), they germinate when subsequently exposed to normal

germination conditions. It is further instructive to experiment with seedlings (say Pisum) whose roots have already attained a length of a few cm., placing them in vessels of water free from air, and isolated from the atmosphere by mercury, and then replacing the water in one vessel with air, that in the other with Hydrogen. In the air the roots of the seedlings continue to grow, but they do not grow at all in the Hydrogen, as can readily be proved by measuring.

In the manner described, it can also be shown that the germination of the seeds will take place in a gas mixture very poor in Oxygen (a mixture, *e.g.*, of air with larger or smaller quantities of Hydrogen).

In order to demonstrate very exactly that the higher plants cannot grow in absence of Oxygen, we proceed as follows. In a test-tube (*R*, Fig. 131), about 15 mm. in diameter and about 60 c.c. in capacity, we fix a pea seedling, raised in sawdust, on whose root ink-lines have been painted as marks. The inside of the test-tube is moistened with a few drops of boiled-out water. The test-tube is closed with a well-fitting two-holed rubber stopper. Through one hole passes the glass tube *g*, through the other the tube *g'*, of which one limb can be dipped into mercury contained in the vessel *Gf*. In order to drive out the air completely, we now pass a stream of Hydrogen through the apparatus for one to two hours, and then fuse up the tube *g* at the point *s*. The Hydrogen may be prepared, say in a Kipp's apparatus (see 102),

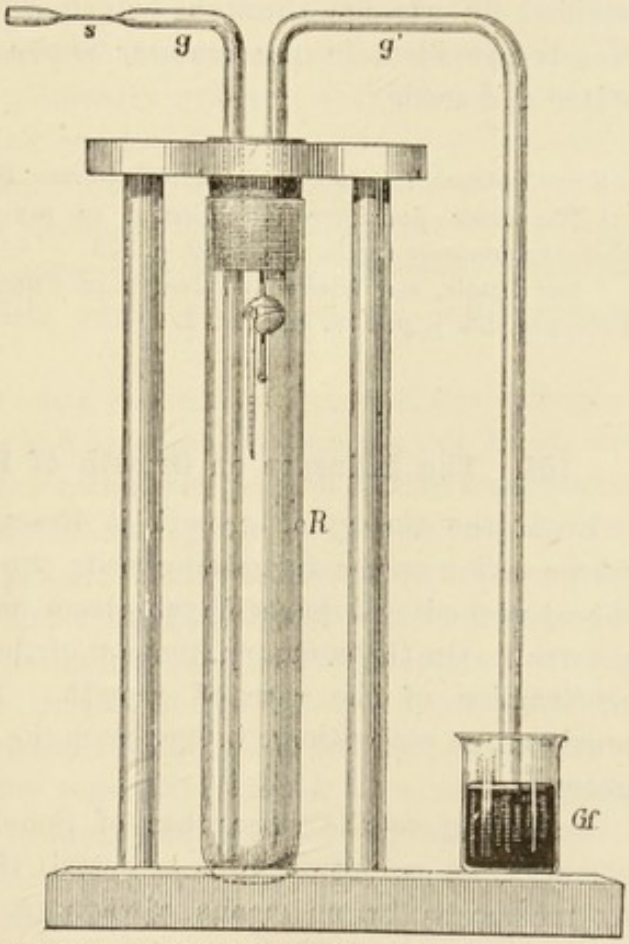

Fig. 131.—Apparatus for proving that roots are unable to grow in absence of free Oxygen.

from Arsenic-free Zinc and dilute Hydrochloric acid. To purify the gas, it is passed through vessels containing solutions of potash and Potassium permanganate. The water intended for diluting the Hydrochloric acid, and for making the purifying solutions, may be boiled before being used and allowed to cool in closed bottles. That our seedlings do not grow in the atmosphere of pure Hydrogen is easily determined by measuring the distance between the marks on the root, at intervals of a few hours, by means of the horizontal microscope (see 153). If we open the apparatus, the growth of the plants at once begins again.

The seeds of different species of plants appear to behave differently in germination towards pure Oxygen.[2] I found that wheat grains germinate as rapidly in pure Oxygen as in atmospheric air. The necessary Oxygen is prepared by heating a mixture of Potassium chlorate and Manganese dioxide in a retort, purified by passage through potash solution, and led into the vessels provided, in the manner above indicated, with air-free water and seeds.[3]

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[1] See Detmer, *Landwirthschaftl. Jahrbücher*, Bd. 11, p. 225.

[2] The older literature is collected in my *Vergleichende Physiologie d. Keimungsprocesses der Samen*, 1889, p. 272.

[3] For details, see Wieler and Jentys in *Untersuchungen aus d. bot. Inst. zu Tübingen*, Bd. 1, p. 216, and Bd. 2.

161. The Influence on Growth of Pressure and Tension.

From the theory of growth it directly follows that pressures, whose action on the turgescing cells consists in a compression of the stretched cell parts (hyaloplasm and cell-wall), must retard growth. On the contrary, tension of these parts will result in an acceleration of the rate of growth. Similarly, pressures and tensions are not without influence on the direction of most vigorous growth.

According to the researches of Scholtz[1] and Hegler,[2] which, however, we cannot examine in detail, the acceleration of growth by tension is by no means always in correspondence with its mechanical equivalent, because if stems, for example, are subjected to tension, their growth at first becomes slower, and does not till later undergo an acceleration corresponding with the mechanical equivalent of the tension. The tension at first acts as a stimulus

to the protoplasm. One may imagine that the cell membranes acted upon by the stimulated protoplasm undergo qualitative changes, which result in retardation of their growth.

If we examine the epidermal cells of the long leaves of many monocotyledons, we find that they are much elongated. This is due essentially to the fact that the epidermal cells of these organs are stretched by the tissue tensions mainly in a longitudinal direction. If we examine a fragment of the epidermis from a leaf of Syringa or some other dicotyledon, it will be seen that the epidermal cells are in the form of polygonal plates, which is certainly related to the fact that the surface development of the leaves takes place in almost the same manner in two directions.

We prepare transverse sections of a twig of Tilia parvifolia, about 5 mm. in thickness. The structure which reveals itself on microscopic examination has already been described in 42. We are here only concerned really with the wood of the vascular bundles. We see that several annual rings are present. The spring wood passes quite gradually into the autumn wood of the same ring, while the spring wood of the next ring is quite sharply separated from the autumn wood of the previous year. The spring wood is specially characterised by the presence of large vessels. Later on these more and more completely disappear. The autumn wood consists entirely of elements with narrow lumen.

Sachs long ago pointed out a connection between the difference in structure of the spring and autumn wood on the one hand, and the varying intensity of the transverse tension during a vegetative period on the other. In the spring the cortex is clearly less stretched than at a later period, when the formation of wood has made further progress. In the spring, therefore, the pressure to which the wood is subjected is less than at a later period, and in these circumstances is to be sought the chief explanation of the fact that the wood elements formed from the cambium cells at the commencement of the vegetative period have wide lumina, whereas later, especially towards the autumn, only elements with small lumen are formed.

We will now see how plant structures growing in thickness are affected when artificially subjected to increase and diminution of pressure. We may employ two to three-year-old branches of various shrubs or trees. Increase of pressure we obtain by winding a piece of string, not too thick, for a few centimetres round the

branch, making the experiment at the beginning of April. The turns of the spiral must be as close together as possible, and the ends of the string firmly tied together. Diminution of pressure we produce by splitting the cortex and bast of a three-year-old branch for a distance of about 3 cm. by six equidistant radial longitudinal cuts. If we examine the branches in August we shall find that the diameter of the ligatured branch under the ligature is considerably less than above or below it, while, on the other hand, the reduction of pressure has induced a not inconsiderable acceleration of growth in thickness in the part of the branch subjected to it. For experiments on the effects of increased pressure on the growth in thickness of twigs, I employed, with very good results, Salix cinerea. The ligature was fixed at the commencement of April, and removed at the commencement of August. The region under the string was, at the end of the experiment, much thinner than the parts of the branch above and below.[3]

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[1] See Scholtz, Cohn's *Beiträge zur Biologie der Pflanzen*, Bd. 4.

[2] See Hegler, the same, Bd. 6.

[3] Exhaustive investigations into the anatomical structure of the wood produced when the pressure is artificially increased or diminished have been made by H. de Vries. See *Flora*, 1872, No. 16, and 1875, No. 7.

162. Influence of Temperature on Growth.

It is a well-known fact that in all plant structures growth by no means proceeds at the same rate at different temperatures. Growth is only exhibited within particular limits of temperature. At certain lower and higher temperatures growth completely ceases, and to prove this for lower temperatures we conduct the following experiment:—A well-developed pea seedling, which has been grown in loose, moist sawdust until its main root has attained a length of 3–4 cm., is placed in the apparatus depicted in Fig. 126, together with some moist cotton wool. I found, *e.g.*, that at a temperature of 20° C. the growth of the root, in the course of eight hours, was 5 mm. I now left the apparatus for twenty-two hours in an unheated room, at a temperature of 1°–2° C. At the end of this time no perceptible growth could be determined. Growth was, however, observable (10 mm.) after the apparatus had been left for the next eighteen hours in a room at a temperature of 15° C.

Accurate researches on the influence of different temperatures on the rate of growth of plants are very tedious, but we must,

nevertheless, endeavour to make ourselves acquainted with the important phenomena in question. For the experiments we employ seedlings. The seeds from which these are to be obtained must be carefully selected. We employ only seeds which are uniform, well developed, and quite ripe. We shall find that these germinate well if the temperature is favourable (about 20–25° C.); on the other hand, we observe at once that the power of germination is no longer so perfect at comparatively low or comparatively high temperatures. The seeds (we experiment with Pisum, Phaseolus, Zea, Cucurbita, etc.) are first laid for twenty-four hours in water, so as to get thoroughly soaked. We now place them in moist garden earth, in such a position that, when their main roots emerge, they can grow straight downwards without needing to undergo any considerable amount of curvature. The soil used is humous garden soil, such as is employed in the cultivation of greenhouse plants. Before being used it is moistened sufficiently to allow of its being rubbed down between the hands into a finely crumbled mass, riddled through a sieve with 1·5 mm. meshes, and then loosely filled into large flower-pots. Finally, the soaked seeds are sown at measured distances from one another, and covered with earth, particular care being taken that they all receive as nearly as possible the same thickness of covering. Each flower-pot is provided with a thermometer, which indicates the temperature of the layer of soil in which the germinating seeds lie. Care also must be taken to replace the water lost by evaporation. The vessels in which the seeds are placed to soak, as also the flower-pots in which the seeds are sown, must be exposed to the particular temperatures whose influence on growth it is desired to determine. If we wish to experiment at temperatures of 25°, 30°, 35°, 40°, or 45° C., we are obliged to place the culture vessels in thermostats, in which the desired temperature can be maintained. We use, *e.g.*, the apparatus depicted in Fig. 76. Observations at 5°, 10°, 15°, 20° C. are often best made without using thermostats, in suitable places unheated, or warmed by good stoves (in summer, *e g.* in rooms with a north aspect, or in cellars). But in the course of the twenty-four hours by no means inconsiderable variations of temperature may take place. Particular attention must be paid to this, and the temperature of the medium in which the germinating seeds are situated must hence be controlled several times in the course of the day. This must naturally also be seen to in using the thermostats. All the

temperature readings must be noted, so that we may be able to calculate the mean temperature.

Each experiment should extend over forty-eight to seventy-two hours, or even longer. It begins the moment the seeds are soaked. At the end of the experiment we take the seedlings out of the soil measure the length of their roots, and so obtain numbers from which we can easily deduce a mean value for the length which a root attains in a particular time at a particular temperature. Thus, *e.g.*, at 25° C., the main root of Zea Mais may attain a length of 30 mm. in the course of forty-eight hours. At 34° C., a root more than 50 mm. in length may be produced in forty-eight hours, while a temperature of 42° C. considerably impairs the growth of the root of Zea. At 15° C., also, the root grows but slowly, and, therefore, even in ninety-six hours, attains no considerable length.

The growth of the plant-cells begins at a certain lower limit of temperature (the temperature minimum), increases in rapidity up to a certain point (the optimum temperature), beyond which the rate of growth again diminishes. The highest temperature at which we find growth to take place at all we term the maximum temperature. The positions of the minima, optima, and maxima of temperature are by no means the same for different structures, which also finds expression, *e.g.* as regards the minimum temperature, in the readily determined fact that many seeds are still unable to germinate at all at a temperature at which the germination of other seeds is possible. At 10° C. the seeds of Cucurbita do not germinate even after a considerable time has elapsed, whereas wheat grains and seeds of Phaseolus multiflorus do germinate at this temperature.[1]

In the following table is given the position of the cardinal points of temperature for germination of some seeds :—

	Temperature :—		
	Minimum.	Optimum.	Maximum.
Triticum vulgare ...	5·0*	28·7	42·5
Phaseolus multiflorus	9·5	33·7	46·2
Pisum sativum ...	6·7	26·6	—
Zea Mais	9·5	33·7	46·2
Cucurbita Pepo ...	13·7	33·7	46·2

The temperatures are expressed in degrees Centigrade.

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* According to recent investigations, the temperature minimum is usually lower.

[1] Literature: Sachs, in Pringsheim's *Jahrbücher*, Bd. 2; Fr. Haberlandt, in *Wissenschl.-praktische Untersuchungen auf dem Gebiete des Pflanzenbaues*; and Detmer, *Vergleichende Physiologie des Keimungsprocesses der Samen*, 1880.

163. The Annual Period.

In many plants the general course of development is interrupted by periods of rest to which their organs are subjected at particular times. Most of our native trees and shrubs shed their leaves in autumn, and the buds previously formed pass the winter in a state of rest, not unfolding till the following spring. Here, undoubtedly, we have to do with a phenomenon originally induced by the alternation of seasons, but which, as it now presents itself, by no means exhibits a direct dependence on external factors. When, viz., the same external conditions constantly act in definite rotation on an individual plant, or on generations of individuals, the plants thereby acquire, in consequence of after-effects, specific peculiarities which may even become hereditary, and these peculiarities then frequently appear so firmly fixed that they very largely determine the entire behaviour of the plant. This must especially be remembered when we attempt to explain why the winter buds of our trees and shrubs do not always open at once when, under suitable conditions, exposed in winter to higher temperature. The resting period of buds is, it is true, primarily induced by the alternation of the seasons, but ultimately it has become in the manner indicated a specific peculiarity of the plant, which cannot be at once laid aside.

If at the end of October twigs of Prunus avium, not too small, are placed with their lower ends in water, and kept in the hothouse, their buds do not burst in spite of the favourable conditions presented, but gradually die; Prunus twigs, on the other hand, brought into the hothouse in the middle of December, flower after about four weeks. If we do not cut the twigs till the middle of January, they flower still more rapidly. Similar results are obtained with branches of other trees, *e.g.* Tilia. Forsythia twigs also sprout very rapidly. I placed some twigs in water at the beginning of December, and they unfolded their leaves and flowers in three weeks.

Moreover the degree of energy with which the winter buds of different kinds of plants develop when shoots cut from them are placed in water and brought into a warm room varies very much.

The buds of willow shoots, *e.g.*, develop very rapidly, as also do those of Syringa (I found that in the hothouse, twigs cut towards the end of February had unfolded their leaves completely in less than fourteen days), while the buds of Laburnum do not succeed in unfolding quite so easily. Experiments made at different parts of the winter, and with different plants, furnish results which are interesting in many respects. We only require for such experiments large branches or twigs, placed with their lower ends in water, care being taken that the air around the plants is not too dry, to ensure which it is generally better to conduct the experiments in the hothouse than in the room.

If we take potatoes into a warm room in autumn, and leave them there in a box, we find that they do not begin to germinate till about the new year. A resting period is thus characteristic of potatoes, as also of buds. Müller-Thurgau [1] has attempted to determine the cause of this resting period, and I [2] have occupied myself with the same question. If after they have been lying in the room for some time, we examine the potatoes for sugar, by rubbing some of them on a grater, adding water to the pulp, and then after a time filtering off the fluid and testing it with Fehling's solution, we shall find no glucose or only traces of it. In January, when the tubers begin to germinate, the quantity of sugar they contain is still very small; it gradually, however, becomes considerable. If in December we treat a small quantity (20 c.c.) of the fluid obtained from potatoes with a little thin starch paste, the presence of diastase cannot with certainty be demonstrated (for method, see 112). Potatoes far advanced in germination, however, certainly do, as I satisfied myself, contain diastase.

From this we may assume that the reason the potatoes do not at once germinate in the autumn, is that they are not able to produce quantities of diastase sufficient for abundant production of sugar. The quantities of sugar formed in the tubers in autumn suffice, it is true, to maintain their respiration; but the sugar does not accumulate to any marked extent in the tissues, and is not sufficient to ensure energetic growth of the buds. Gradually more and more diastase is formed in the tubers; larger quantities of sugar are produced, and germination can begin. It is very probable that the results to which we are led in studying the resting period of potatoes are of significance in explaining the resting period of the winter buds of our trees and shrubs, and this adds interest to the following experiment of Müller-Thurgau.

A few potatoes are dug up in August, and immediately put into a thermostat at a temperature of 0° C. (see 49). After about four weeks the potatoes are placed in flower-pots in loose, moist garden earth, and exposed to conditions favourable to germination, light being excluded. The development of the buds at once begins, while control experiments teach that tubers not previously cooled do not by any means germinate in autumn, but much later. We have, therefore, by cooling the potatoes, eliminated their resting period. It has been shown in 126 that at low temperatures the tubers collect in their tissues considerable quantities of sugar, since under these circumstances the respiration of the cells is very feeble. After the cooling, a fairly large quantity of plastic material stands at the disposal of the buds, and they can therefore rapidly develop. In this connection some observations which I myself made on Pavia twigs are also of interest. Pavia twigs, cut in the middle of January, placed with their lower end in water, and left in the hothouse, unfolded their buds in the middle of March. Twigs of Pavia cut at the end of October, and taken into the hothouse, did not open their buds till after the middle of March. The comparatively rapid development of the buds not placed in the hothouse till January is perhaps due to their being able, while in the open, to accumulate sugar in their tissues, in consequence of the lower temperature to which they were then exposed. Twigs removed to the hothouse as early as October have also perhaps produced sugar; but this, if not very abundant, would be used up in respiration, so that the buds would be unable to unfold till March. At this time certainly a specially energetic production of diastatic ferment takes place in the twigs; the quantity of sugar now formed is sufficient not only for the maintenance of respiration, but also to enable the buds to begin to develop.[3]

The following observation is also of interest:—The willow (Salix fragilis) mentioned on p. 8, which was reared by water culture, and stood all the winter in a warm room, kept perfectly healthy, but did not unfold its buds till March 26, 1895. Also twigs cut from this willow and placed in water have only formed new shoots within the last few days. On the other hand, shoots removed in the middle of December from plants growing in the open, when placed in the warm room in water, produced roots and new shoots in the course of four weeks.

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[1] See Müller-Thurgau, *Landwirthschl. Jahrbücher*, Bd. 11, p. 813.

[2] See Detmer, *Pflanzenphysiologische Untersuchungen über Fermentbildung*, etc., Jena, 1884, p. 41.

[3] The results of A. Fischer's investigations also favour the views above brought forward (see *Jahrb. f. wissenschl. Bot.*, Bd. 22, pp. 127 and 154). Müller-Thurgau has recently taken a somewhat different view of the cause of the resting period in plants (*Landwirthschl. Jahrb.*, Bd. 14, p. 878). See further Askenasy, *Botan. Zeitung*, 1877.

164. Growth of Plant Structures in Constant Darkness.

Vigorous growth in constant darkness can naturally only take place in structures which, even under these conditions, have at their disposal considerable quantities of plastic material. Hence seedlings are especially suitable for the following experiments, because in their receptacles of reserve material larger or smaller quantities of plastic substances are in fact always present. To compare in a general way the behaviour of plants when growing in constant darkness, with their behaviour when growing under normal conditions of illumination, we place a few soaked seeds of Pisum, Phaseolus, and Cucurbita in large flower-pots filled with moist garden earth. Some of the flower-pots are placed at the window, so as to be subject to alternation of day and night; others stand immediately by them under a large cardboard box covered with black paper. It is well to conduct the experiments in a room in which the plants are only exposed to diffused light, since under the influence of direct sunlight the air in the cardboard box would rapidly assume a very high temperature. It soon appears that the two sets of plants, those developing in darkness on the one hand, and those growing under normal conditions of illumination on the other, present a very different appearance. Leaving entirely out of consideration the absence of green colour in the plants growing in the dark, we shall find, *e.g.*, that in Cucurbita, the hypocotyl has, in darkness, attained a very considerable length, whereas in the illuminated plants it is still comparatively short. The cotyledons of the shaded plants are, on the contrary, neither so broad nor so long as those of the plants growing in the daylight. By accurate measurements (several plants must always be examined in order to obtain a trustworthy average) we can prove this in detail. In Fig. 132 is represented the aerial part of an etiolated seedling of Cucurbita, and in Fig. 133 the corresponding portion of a normal seedling. By growing seed-

lings of Phaseolus in light and darkness respectively (see Figs. 134 and 135) we can also readily make out that the hypocotyl

FIG. 132.—Aerial part of a seedling of Cucurbita grown in the dark.

FIG. 133.—Aerial part of a seedling of Cucurbita grown under normal conditions.

remains very short even in darkness, while the epicotyl reaches a much greater length than in light. The leaf-stalks of the primordial leaves are longer in darkness than under normal conditions; the blades of the leaves, on the other hand, only attain their normal form and size in the light. Seedlings of Pisum and Vicia behave like Phaseolus seedlings. The seedlings of Tropæolum majus also

offer excellent material for observation. In many monocotyledons (Zea, Triticum) we find on investigation that the leaves of seedlings grown in continuous darkness, as compared with leaves of the same age which have developed in the light, attain a considerable length, but remain narrow.[1]

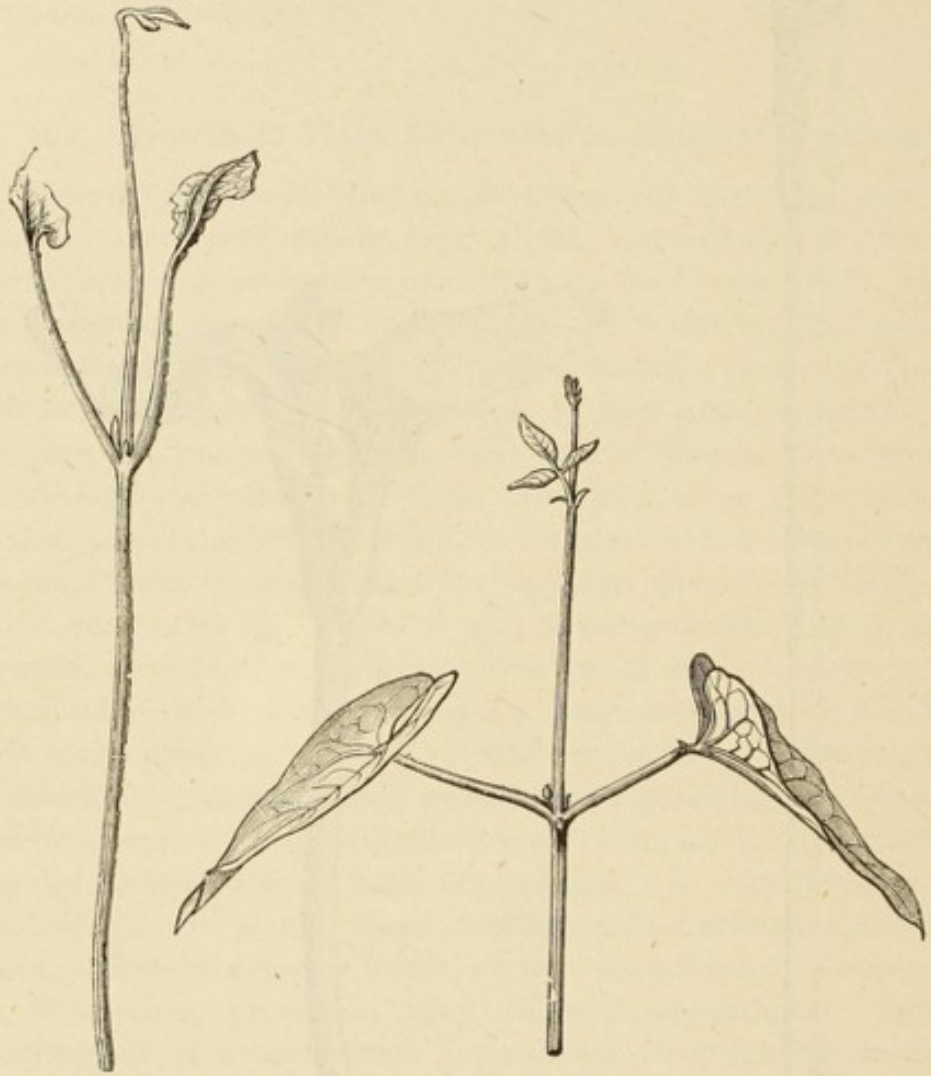

FIG. 134.—Aerial part of a seedling of Phaseolus grown in the dark.

FIG. 135.—Aerial part of a seedling of Phaseolus grown under normal conditions.

It is of interest that not only do plants grown under ordinary illumination on the one hand, and in complete darkness on the other, exhibit considerable differences in their entire development, but that similar differences also clearly appear in the cultivation of plants in light of greater and less intensity.[2] To demonstrate this I have employed wooden boxes 55 cm. high and of about 680 sq. cm. area of base for the reception of the culture vessels and

plants. The boxes were blackened inside. Plates of glass could be slipped in to form the front. One box may be provided with a sheet of ordinary glass, another with a single sheet of opal glass, a third with two sheets, and a fourth with four sheets of opal glass. Plants are also, for comparison, grown in complete darkness. We place the boxes in front of the window in a room with a north aspect. Using beans, we shall soon observe that the plants which receive most light produce the shortest stems and the largest leaves. With diminishing intensity of light, due to interposition of the sheets of opal glass, the length of the stems increases, while the size of the leaves diminishes.

Various plants are able to develop in constant darkness not only numerous leaves and long stems, but also flowers. Thus if Hyacinth bulbs are germinated in the so-called Hyacinth glasses in continued and complete absence of light, they will actually flower. I satisfied myself that the flowers, as regards form and colour, develop quite normally in absolute darkness, behaving therefore in a manner different from most stems and foliage leaves.[3]

It is further of interest to make the following experiment. We raise plants of Phaseolus multiflorus in flower-pots under ordinary conditions. When the primordial leaves have developed normally, and the internodes following the epicotyl are rapidly elongating, we expose the apex of a plant to darkness, leaving the rest of it in bright daylight. This is effected as follows. A stand carries a large metal ring, on which rests horizontally a sheet of thick cardboard with a hole in the middle of it. The apex of the plant is passed through this hole, and tightly fixed in it with cotton wool. Finally we place over the bud a tall cardboard cylinder covered with black paper, with its edge resting on the sheet of cardboard. In the course of two or three days we shall find that the new internodes produced thus in darkness are abnormally long, while the leaves remain small.*

If this experiment is to succeed well, the plants must be put in a place where, at any rate for a good part of the day, they receive

* This result is not in complete accordance with certain statements of Sachs, *Lectures on Plant Physiology*, p. 532. In the experiments of Sachs, however, the conditions were not the same as in mine, since his research plants possessed numerous leaves, exposed to the light and vigorously assimilating. The darkened leaves attained a considerable size, and the like may also under similar conditions take place in Phaseolus. The whole question, however, demands further experimental examination. Consult Frank, *Lehrbuch der Botanik*, Bd. 1, and Amelung, *Flora*, 1894.

direct sunlight, *e.g.* at a window. The part of the plant under the box is prevented from becoming too warm by means of suitably placed screens. In my experiments I found that the portions of bean stems growing in the dark were able to twine round a support.

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[1] See Sachs, *Botan. Zeitung*, 1863, Beilage.
[2] See Detmer, *Versuchsstationen*, Bd. 16.
[3] See also Sachs, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 3, p. 372.

165. The Causes of Etiolation.

It is a fact that many kinds of plants, when grown in absolute darkness, produce abnormally long stems and small leaves. The first question which presents itself is whether the peculiar formation of etiolated plants is not perhaps due to the arrest, in darkness, of the assimilatory activity of their leaves. To obtain an answer to this question we conduct the following experiments with seedlings of Raphanus sativus.[1]

Raphanus seeds are soaked in water, and then laid on coarse sand contained in two small flower-pots, and watered with dilute food solution. Each flower pot is then placed in an arrangement such as was described in 16, and represented in Fig. 18. One apparatus is exposed to bright diffused daylight, the other is placed close to it, under a cardboard box covered with black paper. The seeds soon germinate, and while the shaded seedlings produce long hypocotyls and cotyledons of small width and length, the illuminated seedlings, which have become green in colour, present a perfectly normal appearance. These last however, although they possess chlorophyll, cannot assimilate, for they are surrounded by air devoid of Carbon dioxide, and therefore failure of assimilatory activity cannot be regarded as the cause of the peculiar formation of etiolated plants.*

To obtain a closer insight into the causes of etiolation, it is important to make the following experiment. A number of Raphanus seeds are selected as nearly as possible of the same size. We determine the weight of each individual seed, and only use for the experiment such as are approximately of the same weight. After

* But light may be of importance for the formation of specific substances which are necessary for normal development of the leaves. These bodies may provisionally be designated leaf-forming substances.

soaking them we lay the seeds on sand moistened with food solution, in small flower-pots. Each pot is provided with four or five seeds. The cultures are placed, as above, in air free from Carbon dioxide, one of them exposed to the light, the other in darkness. After a few days, when germination is fairly advanced, we remove them carefully from the sand, and divide them up into their separate organs. Seedlings which have not developed in a perfectly normal manner are rejected. For our purpose we need only proceed to examine the hypocotyl and the cotyledons. We determine the weight of these organs, dry them in small glasses at 100° C., and then again weigh them. With care we obtain the following results. The hypocotyls of the plants grown in the light are absolutely poorer in dry substance than those of the plants grown in the dark. The former contain a smaller percentage of water than the latter. The cotyledons grown in light are absolutely richer in dry substance than those developed in darkness. These latter contain a smaller percentage of water than the former.*

The problem now is to explain clearly why etiolated internodes are usually considerably longer than normal ones, and why the leaves in absence of light are, for the most part, very backward in development. As regards the abnormal elongation of etiolated internodes, it is first to be pointed out, that the membranes of the elements of their tissues (epidermis, collenchyma, bast, wood) remain in an early stage of development, and do not attain their normal thickness. This can be proved by comparing epicotyls of Phaseolus plants grown in the light and in the dark respectively, as regards the structure of their wood elements. The tissue of etiolated internodes must, consequently, be more extensible than that of normal ones, and each individual cell of an etiolated stem will also be able to grow more rapidly than the corresponding cell of internodes developed under the influence of alternating day and night, since its membranes oppose only a comparatively small resistance to the osmotic pressure. It may here also be pointed out, and this stands in immediate relation to what has been said, that, as Kraus first indicated, the intensity of the tissue tension (longitudinal tension) in etiolated internodes, is considerably less than in normal ones. We can satisfy ourselves of this fact by making comparative experiments in the manner described in 149, on the tissue tension of normally developed and etiolated epicotyls

* I have already shown elsewhere (*Versuchsstationen*, Bd. 16, p. 212), that etiolated plants are richer in water than plants grown in the light.

respectively of Phaseolus. We examine plants of the same age and in vigorous growth.

A further reason for the rapid growth of etiolated internodes appears in certain cases to lie in the fact that a greater osmotic pressure is set up in their cells than in the cells of normal organs. Some observations of Wiesner and H. de Vries[2] indicate that etiolated structures are relatively rich in organic acids, and as these bodies are of great significance in the development of turgor, it should be of interest to investigate the matter more closely. Suitable research material would be found *e.g.* in the epicotyls of Vicia sativa or Phaseolus. It would only be necessary to compare the acidity of organs grown in the dark and of organs grown in the light. (For the method see 130.)

Other observations, however (see Pfeffer, *Handbuch*, Bd. 2, p. 145; de Vries, *Jahrbücher f. wissenschl. Botanik*, Bd. 14, p. 561, and Wortmann, *Botan. Zeitung*, 1889, p. 296), tend to show that the osmotic pressure of the cells of etiolated stem structures is not greater than that of normal ones. The whole question requires thorough investigation.

The active growth of etiolated stem structures is due, therefore, to great turgor-extension of their cells. This in turn is due to increased osmotic pressure of the cell-contents (?), accompanied by comparatively slight power of resistance on the part of the cell membranes. From what has been said it may be imagined that the individual cells of etiolated stems are of greater length than the corresponding cells of normal internodes. And in fact this is the case. I have, for example, determined by means of a stage micrometer the length of the pith cells from the middle of normal and etiolated epicotyls respectively of Phaseolus. The former were about 0·2 mm. in length (it is always necessary to measure a number of the cells, and take the mean value), the latter two to three times as long.[3]

As to why the leaves of most dicotyledons remain so very small in darkness, only the following need here be noted. In darkness the processes which bring about a vigorous surface growth of the cell membranes do not take place. What these processes are it is not exactly known (see, however, the footnote on p.408). It is only certain that these processes—and with them the surface growth of the leaf-cells—can take place when the plants are struck by the rays of light, even if only transitorily. An experiment of Batalin's, which may easily be repeated, clearly shows this. We grow

seedlings of Phaseolus in flower-pots in darkness. When the primordial leaves have developed to a certain extent, we select two plants, *a* and *b*, whose leaves are as nearly as possible of the same size, and measure their length and breadth. *a* is still left in darkness; *b* is exposed for about two hours each day during eight days to weak diffused light, but otherwise also remains in darkness. The leaves of *b*, being only illuminated each day for so short a time, do not become green. The leaves of *a* remain small, while those of *b* grow considerably.[4]

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[1] See Godlewski, *Botan. Zeitung*, 1879.

[2] See H. de Vries, *Botan. Zeitung*, 1879, p. 852.

[3] See G. Kraus in Pringsheim's *Jahrbücher*, Bd. 8. Increased multiplication of the cells, as well as increased elongation of the cells, plays a part in the production of etiolation.

[4] For the literature on etiolation, see Detmer, *Vergleichende Physiologie des Keimungsprocesses d. Samen*, 1880.

166. The Influence of Light on Growth.

It is well known that light exerts a retarding influence on the growth of the most various plant structures. To prove this fact we make the following observations. A considerable number of well-developed pea seeds are soaked and germinated in a box filled with damp sawdust. When the main roots of the seedlings have attained a length of 2 cm. we remove them from the sawdust, and mark them, in the usual manner, at a distance of 10 mm. from the root tip, with fine ink-lines. Special care must be taken that only very similar and perfectly normal seedlings are employed for further investigation. The culture of the plants is continued in glass cylinders, about 25 cm. deep and 10 cm. in diameter, which are provided with suitable wooden covers pierced with a number of holes, and which are filled with spring water. We take two such cylinders. Each is fitted up with a fair number of the seedlings (perhaps ten to fifteen), which we fix in the holes of the cover by means of cotton wool, and in such a way that their roots dip into the water. The roots in one of the culture cylinders are left exposed to the light; it is desirable to place a mirror close behind the cylinder and parallel with the window, or to rotate the cylinder slowly on a clinostat, so as to prevent any heliotropic curvature of the roots. The other cylinder is covered with black paper, so that the light cannot reach the roots. We conduct the observations in summer, in a room with a north aspect, and at as high a

temperature as possible. From time to time, say every twenty-four hours, we determine the aggregate growth of all the roots in each cylinder, and we shall arrive at the result that the growth is less in the light than in the absence of light.

If plants are exposed to alternation of day and night, the temperature and moisture being kept as constant as possible, it will be found that generally the growth of their organs increases from evening to morning, but diminishes from morning to evening. This daily period of growth is the result of the variation in the conditions of illumination during the twenty-four hours. In the daytime the light retards the growth; the darkness during the night accelerates the rate of growth. Sachs[1] has, by means of the auxanometer, determined the fact that there is a daily period of growth in the internodes of various plants, and Prantl[2] has similarly demonstrated the existence of a daily periodicity in the growth of leaves. The experiments necessary are very tedious. It is simplest to demonstrate the daily period of growth in leaves. We employ for the purpose the methods described at the end of 154. The plants (Cucurbita or Nicotiana) are placed under bell-glasses in a room with a north aspect, and exposed to the alternation of day and night, the temperature being kept very high and as uniform as possible. From time to time (*e.g.* every three or four hours) we measure with a millimetre scale the distance between the apex of the leaf and the mark at its base, taking care not to drag the leaf too much in laying it out flat. It then appears that the growth is greater during the night than in the day. When darkness comes on in the evening, the growth of the leaves is not considerably accelerated at once, but quite gradually, so that the maximum of the daily growth falls in the morning hours. Similarly the daylight does not at once reduce the rate of growth of the leaves to the minimum; the minimum rate of growth does not appear until evening.

Similar results are obtained if we determine the growth in width of the leaves as well as their growth in length. The necessary marks are made on the edges of the leaves, in the neighbourhood of the greatest diameter, and the distance between them is determined at intervals of three or four hours.

In researches on the daily period of growth of internodes, it is very convenient to employ shoots of Dahlia variabilis grown in the light. The tubers are placed in the soil, which is contained in very large flower-pots, a good time before the beginning of the

investigation, so that the plants are already rooted. Several days before the beginning of the experiments the soil in the flower-pots is thoroughly moistened, and then in order to prevent it from drying up, which would interfere considerably with the course of the observations, the pots are put into a zinc receiver, the cover of which is in halves. We employ an auxanometer (see 153). The thread may be fixed say below the leaves of the third, fourth, or fifth internode. These leaves, and also those below them, it is then advisable to cut off close to the base, and always determine the total growth of the parts of the stem lying below the point of attachment of the thread, that is, *e.g.*, of the second and third or fourth and fifth internode. The plants are to be screened from direct sunlight; they must therefore be placed *e.g.* in a room with a south aspect, at a sufficient distance from the window. Mirrors serve to prevent heliotropic curvatures (see 153). The temperature and hygrometric state of the air are to be determined by means of wet and dry bulb thermometers hanging free in the neighbourhood of the research plants (see 76). In order to keep the air in the laboratory nearly uniformly moist, the floor must be sprinkled several times a day with water. Attention must also be paid to the effect of overclouding.

The interpretation of the numerical data afforded by the temperature readings and the measurements of the hourly growth is not quite easy. The peculiar relationship between amount of growth and conditions of illumination stands out fairly clearly, if we simply calculate from the hourly values the increments of growth and mean temperatures for periods of three hours. The relationship between growth and conditions of illumination comes out most clearly of all if the results of the observations are represented graphically. The curves are plotted in the usual manner. Sachs (*Arbeiten d. bot. Inst. in Würzburg*, Bd. 1, pp. 126, 185, and 192) has drawn attention to some points deserving attention, which for want of room we shall here pass over. The curves teach that from morning till evening the growth becomes less, while from evening till morning it increases. This stands out clearly even when the temperature in the night is somewhat lower than in the daytime.

Lastly, it is very instructive to observe the growth of the sporophores of Mucor in light and darkness. We proceed exactly as described in 153. The bell-glasses, under which the Mucor plants grow, are alternately covered with a cardboard case, and left un-

covered. During the period of darkness, the growth of the sporophores is more active than in the period of illumination (see the following table, and the corresponding graphic representation). It is found that exposure to light retards the growth in this case immediately, while darkness immediately accelerates it.[3]

Time.	Growth per hour.	Temperature in degrees Centigrade.
8–9, Forenoon	2·70	22·9
10, ,,	2·70	24·3
11, ,,	2·30	26·0
12, ,,	2·90	25·0
1, Afternoon	2·70	25·8
2, ,,	3·20	25·8
3, ,,	3·50	25·2
4, ,,	2·90	25·0
5, ,,	3·20	25·1
6, ,,	2·80	25·3

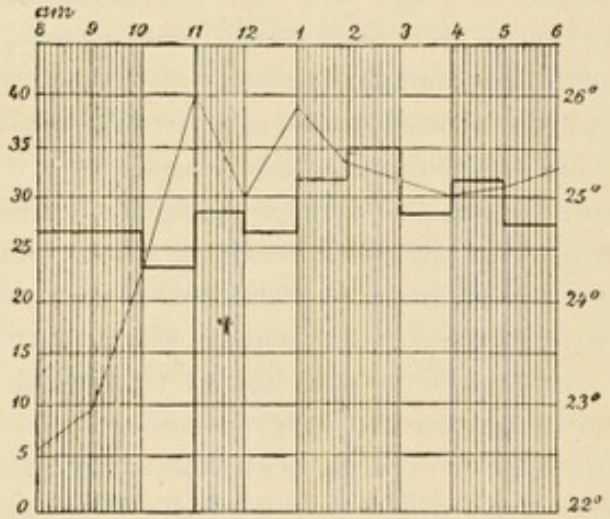

FIG. 136.—The thick line indicates the alternations in the rate of growth of a Mucor tube corresponding with alternations of light and darkness. The fine line is the temperature curve. (After Vines.)

[1] See Sachs, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 99.
[2] See Prantl, the same, p. 371.
[3] See Vines, the same, Bd. 2, p. 133.

167. The Influence of Illumination on the Germination of Potato Tubers.

In the autumn, or in the course of the winter, we lay a number of potatoes in a box, and cover it with a sheet of cardboard so as

to screen them from the light. Other potatoes we place in a box covered with a sheet of glass. The two boxes are placed in front of the window in a heated room with a north aspect. No water at all is supplied to the tubers. During the winter the potatoes germinate, but whereas the shaded ones produce fairly long shoots, the shoots from the illuminated ones remain short and compact in appearance. We may continue the investigation on into the summer, and it is always found that the development of the first internodes of the shoots arising from the potatoes can only proceed normally in darkness (under ordinary conditions in the soil). If now and then we rub down on a grater one tuber from each series, treat the pulp in each case with water, filter, and test the filtrate with Fehling's solution for sugar, we shall find that the one which had been shaded contains a large quantity of sugar, while that which was illuminated contains none at all or only traces. With this want of suitable plastic material in the illuminated tubers is clearly connected the weakly growth of their shoots. I was the first to establish the fact[1] that potato tubers germinating in the light do not contain sugar; Ziegenbein (Pringsheim's *Jahrbücher*, Bd. 25) pursued the matter further.

It is further noteworthy that potatoes placed under the influence of the light gradually become green. If we examine under the microscope delicate transverse sections from a potato which has become green, we find immediately below the skin cells which contain chlorophyll bodies with starch enclosures. These chlorophyll bodies are produced under the influence of the light from colourless amyloplasts which the potatoes contain.

The appearance of the shoots of germinating potatoes is quite different if they are supplied with water. We fill a few plates with moist sand, and keep the sand constantly moist. A few potatoes are pressed into the sand upright, with their morphological base downwards. Some of the plates are covered with a large bell-glass, the others are placed in a large zinc box. The shoots of the illuminated specimens, especially their first internodes, develop as short thick structures provided with numerous scale leaves, but furthermore roots are formed, which penetrate into the sand. Here again the shoots of the tubers kept in the dark attain a considerable length while remaining small in diameter, and their root primordia burst out. In moist air, therefore, many roots always develop on the potato shoots; in dry air they do not develop at all, or only to a very limited extent. Moreover, moist

air is generally more favourable to the development of the stolons than is dry air.

We lay potatoes in moderately moist soil, so that they are completely covered by it. The large flower-pots containing the soil are placed in a warm room under a zinc receiver. On germination the potatoes produce very long shoots with small leaves. Furthermore many aerial roots develop, and soon stolons also, as axillary shoots, which in the moist air bend downwards, and frequently swell at their ends into small tubers. Frequently, however, the tubers are unstalked and seated directly in the leaf axils. The development of the plants under the conditions described is not always exactly the same; the variety of tuber is a matter of importance in this connection.[2] Winter buds of Fagus, according to recent researches, are said to burst only in the light. My experiments on the subject are not yet completed.

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[1] See Detmer, *Pflanzenphysiologische Untersuchungen über Fermentbildung und fermentative Processe*, Jena, 1884, p. 34. In my experiments no water at all was supplied to the potatoes. The water requisite for the growth of the shoots streamed to their cells from the tissue of the tuber.

[2] For much detailed information see Vöchting, *Bibliotheca botanica*, Cassel, 1887, Heft 4.

FIFTH SECTION.

Movements of Irritation.

I THE MOVEMENTS OF IRRITATION OF PROTOPLASMIC STRUCTURES.

168. Protoplasmic Movements.

As the first object for examination, we take Nitella, an alga which is fairly common in stagnant waters poor in lime, employing the younger internodes for microscopic observation. Without going further here into the well-known peculiarities of the elongated cells of Nitella, it may be remarked that the ectoplasm (*Hautschicht*) or hyaloplasm is specially well developed in these cells. This ectoplasm, and likewise the chlorophyll grains embedded in it, are motionless; in the granular layer, on the contrary, very active movement can be perceived. We have here to do with typical rotation, for the stream returns into itself. The ascending part of the stream is separated from the descending part by the indifferent strip.

If we mount leaves from the bud of Elodea canadensis in a drop of water, and examine under the microscope, we recognise without any trouble the parietal layer of protoplasm, the protoplasmic bands traversing the cell-sap, the nucleus, and the chlorophyll grains. We also at once see that movements are taking place in the protoplasm, which are readily observable owing to the change in position which the chlorophyll grains undergo. The protoplasmic movement in the cells of Elodea leaves is sometimes more of the nature of rotation, sometimes more of the nature of circulation.

In this latter the currents, which may occur both in the parietal layer and in the protoplasmic bands, take the most various directions; often the currents in one and the same band even are differently directed. Redistributions of mass take place in the protoplasm; some strands become thinner, others completely

disappear, or new strands originate, and so forth. Excellent objects for the study of the circulation of protoplasm are staminal hairs of Tradescantia (*e.g.* T. virginica) taken from opening flowers, and examined in a drop of water under the cover-glass, as also are the hairs of the young shoots of Cucurbita Pepo.

In the hairs of Tradescantia, Cucurbita, etc., primary protoplasmic movement is exhibited, *i.e.* the circulation takes place in perfectly intact cells, and can hence be at once observed immediately after setting up the preparation. In the leaf-cells of Elodea (we select examples in which we can see the bands well, and in which not too many chlorophyll grains are present on the near walls), immediately after the leaves have been cut off, the movement is only feeble, and often only to be observed with difficulty. Gradually, *e.g.* after half an hour or an hour, it becomes more vigorous, and then even tears away many of the chlorophyll grains.

Very generally, in perfectly intact plant-cells, the protoplasm appears at rest; the movement only takes place as the result of stimuli due to the injuries which are unavoidable in making the preparations. If we remove strips of epidermis from maize plants 6 cm. in height, and examine them as is best in 4 p. c. cane-sugar solution (in order to avoid the injurious effect of the water), we perceive no protoplasmic movement. Movement is not clearly observable till after a quarter or half an hour. Here, then, as in very many other cases, the primary protoplasmic movement is entirely wanting. The secondary movement which sets in is the result of injury.[1]

As to the causes underlying protoplasmic moments little is yet known. In any case, however, when protoplasmic movements are exhibited, a whole series of different processes are concerned, and in different cases perhaps the chemical and physical antecedents are not always the same. Berthold[2] has attempted to make the different forms of movement in protoplasmic masses intelligible by reference to the movements exhibited under certain conditions by dead particles.

It is instructive to prove that inanimate substances are often capable of movements which bear a certain resemblance to protoplasmic movements, a fact which, with many other circumstances, must undoubtedly receive consideration in founding a future theory of protoplasmic movements.

On a dry sheet of glass, resting on white paper, we place with

a glass rod a few drops of not too concentrated alcoholic fuchsin or methyl violet solution. The drops do not spread themselves at their edges uniformly over the glass, but there form, now at one point and now at another, protrusions of the fluid, and we involuntarily recall amœboid protoplasmic movements We pour distilled water into a carefully cleaned glass dish, and drop small fragments of camphor on to it. The particles of camphor, as they very gradually dissolve in the water, fall into very active movement, which I have often seen to continue for hours.

If by means of a glass rod, we bring a trace of olive oil on to the water on which the camphor is moving, the movement rapidly ceases. The spreading oil, viz., raises very considerably the surface tension of the water, and hence the phenomenon described. If a drop of cod-liver oil is treated with 0·25 p.c. soda solution, very interesting phenomena of spreading appear. There are changes in the surface tension which appear quite sufficient to account for the movements in question, and changes in tension also without doubt play a great part in connection with protoplasmic movements. Very thorough study is still required, however, in order to comprehend in detail the complicated phenomena as they are exhibited in the living cells.[3]

Temperature has a very important influence on the rate of movement of the protoplasm. At a low temperature the protoplasm moves slowly. With rising temperature the rate of movement increases, until the optimum temperature is passed (the optimum temperature for protoplasmic movement in the leaf-cells of Elodea, for example, lies, according to Velten,[4] at 36° C.), when it again diminishes. It is instructive to determine by observation that at a particular temperature, not far removed from that at which the cells are killed, the protoplasm passes into a state of transient heat-rigor.

We warm some water in a porcelain dish on the water-bath. Into the water dips a thermometer. We now remove a strip of epidermis from a young part, say a young leaf-stalk, of a plant of Cucurbita Pepo, observe the occurrence of circulation in the protoplasm of the hair-cells, note carefully some of the hairs, and then with the forceps immerse the strip of epidermis in the heated water close to the bulb of the thermometer. If the epidermis is left for two minutes in water at a temperature of 46° C., it will be seen on examination under the microscope that all movement of the protoplasm in the hair-cells has ceased. The protoplasm

has gone over into a state of transient heat-rigor. After one to two hours, however, at a lower temperature, protoplasmic movement is again exhibited.[5]

Protoplasmic movement is arrested at low as well as at high temperatures. In many cases the temperature at which the protoplasm, while not yet killed, is still, for the time being, in a state of cold-rigor, is to be sought at 2° or 4° C. If, however, shoots of Cucurbita are kept in a place at a temperature of 10° C. for some time, in fact till they cool down to this temperature, the protoplasm in the hairs is already in a state of cold-rigor. With rise of temperature the circulation of the protoplasm begins again.

By means of special apparatus for regulating the temperature, we can determine that with rise of temperature from a minimum, the movement of the protoplasm becomes more active, proceeds most actively at a particular temperature (*e.g.* about 35° C. in the hairs of Cucurbita), and at temperatures above this again falls off.

A very useful arrangement of this kind has been constructed by Pfeffer,[6] but Sachs' apparatus,[7] depicted in Fig. 137, may also be employed. The size of the warm chamber must correspond with that of the microscope. The case, approximately cubical in form, has double walls below and at the sides made of sheet zinc, and separated from each other by an interval of 25 mm., the space between them being filled with water. The case is quite open above, but there is an opening in the front wall, which is closed by a well-fitting but not otherwise fixed sheet of glass. The window, *D*, is of such size, and so situated, as to allow sufficient light to fall on the mirror of the microscope standing within the case. The height of the case is so arranged that the top of the double wall is on a level with the bridge of the microscope, and it is provided with a thick cardboard cover, cut out so as to fit exactly round the bridge. Close to the tube of the microscope there is a round hole in the cover, in which a small thermometer fits tightly, its bulb hanging beside the objective. The box is painted on the inside with black varnish, and a piece of cardboard saturated with water is placed under the foot of the microscope, which is thus made to stand more firmly. The moistened cardboard cover also serves the purpose of keeping the air moist round the object under examination. This latter is easily focussed by means of the focussing screw which projects through the cover two side openings, of which the figure shows one, *F*, render it

possible to move the slide when necessary by means of the forceps. It is still more convenient to attach the slide to a wire which passes through a cork fitting into the opening *F*. The warm

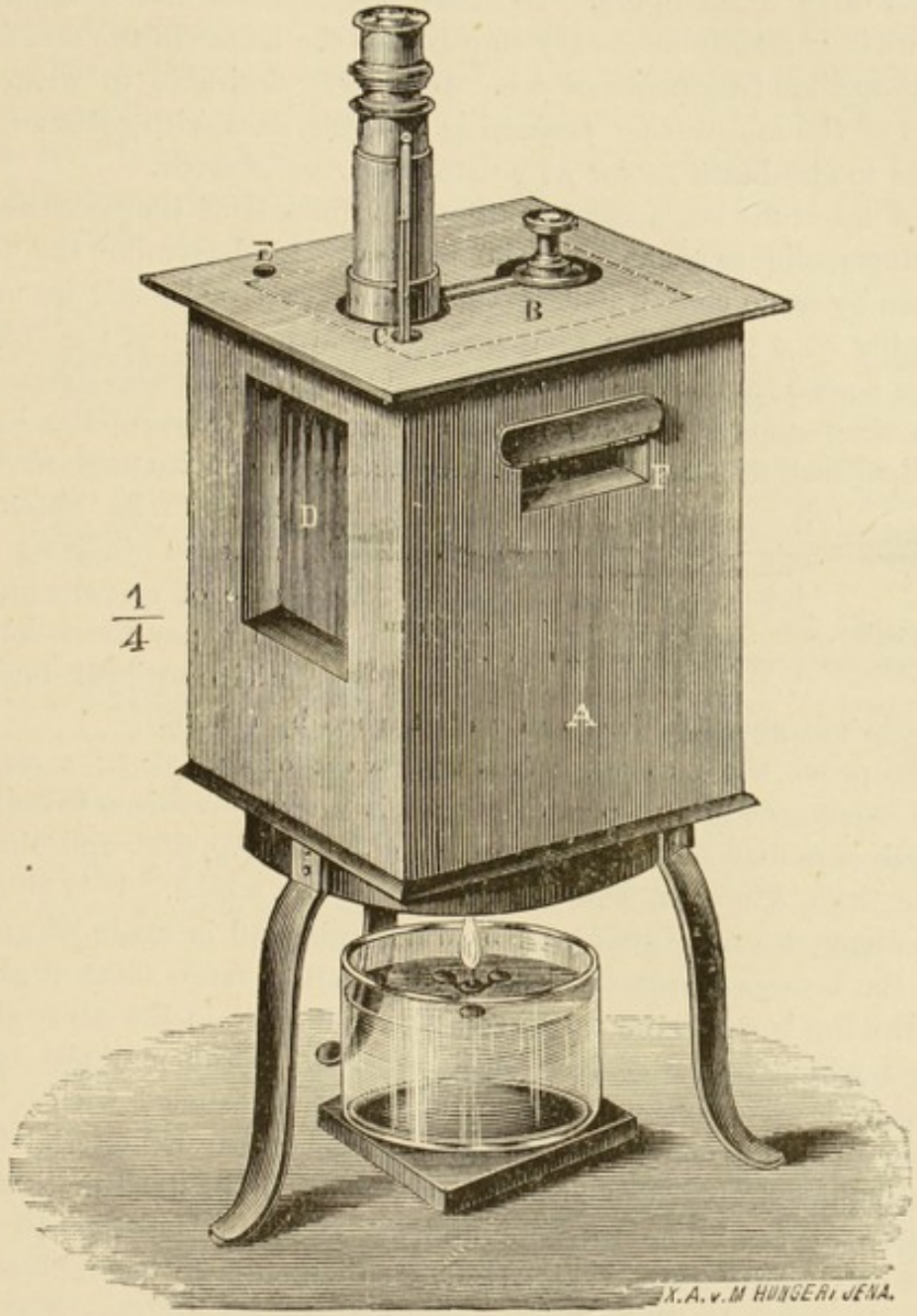

FIG. 137.—Warm chamber. (After Sachs.)

chamber is to be obtained from Leitz in Wetzlar. If it is desired to work at high temperatures (we cannot go beyond 50° C., or the objective will suffer), the water-jacket of the case is heated by means of a spirit-lamp placed below; when the temperature has reached somewhere about the desired height, we replace the spirit-

lamp by an oil float-lamp, such as is represented in the figure, and wait till the temperature becomes constant. To get higher or lower temperatures, it is sufficient to put into the lamp one, two, or three floats with night-lights. To make observations at low temperatures, it is merely necessary to put into the water in the box from time to time fragments of ice. It is very desirable to wrap the part of the microscope projecting from the case with cotton wool, so as to eliminate as far as possible sources of error.

In accurate researches as to the influence of temperature on protoplasmic movement, it is necessary to determine the time taken by some constituent of the cell, *e.g.* a chlorophyll grain, in passing at a particular temperature from one edge of the field of view across the middle to the other edge. We may also determine the time required by a chlorophyll grain to traverse the longitudinal wall of a cell not extending across the entire field of view. It is to be observed, that in judging the rate of movement, we only pay attention to chlorophyll bodies whose movement is free and undisturbed by others.

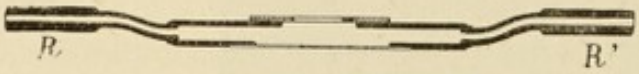

FIG. 138.—Gas chamber for employment in microscopical researches (represented in section).

To prove the important fact that want of free Oxygen arrests the movement of the protoplasm, it is very convenient to employ a gas chamber such as that represented in Fig. 138, and obtainable from Albrecht, Tübingen, at a price of 15 mks. It is about 7 cm. long, 4·5 cm. broad, and 5 mm. high, and is made of metal. At the bottom is cemented air-tight a rather large sheet of glass; at the top is a circular opening. Over this is laid the cover-glass, from which hangs a drop of fluid carrying the material to be observed. The bottom of the chamber is moistened with a thin layer of boiled-out water, to prevent the research material from getting dried up. The cover-glass can readily be fixed air-tight by means of fat. The tubes *R* and *R'* serve to lead gas to and from the preparation. The Hydrogen which is to be employed is prepared and purified in the manner described in 160. The gas chamber is placed on the stage of the microscope. We select for examination say staminal hairs of Tradescantia, or hairs from the young leaf-stalk of Cucurbita Pepo. It is also convenient to experiment with infusoria. We easily obtain them by flooding hay with water, and allowing to stand for about eight days. The fluid then contains chiefly numbers of Paramecia, whose elongated bodies

are provided with cilia. We also find Vorticellæ. The material (hairs or infusoria) having been transferred to the hanging drop, and the occurrence of movement having been proved, the current of Hydrogen is at once started. I have often found it necessary to continue the stream of gas for some time (sometimes for several hours) before the movements ceased. The streaming of the protoplasm, and the free locomotion of the organisms, continues to take place even in presence of minimal quantities of Oxygen. When the protoplasm has at last become motionless, the state of asphyxiation induced can be relieved again by passing a current of air.[8]

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[1] See Hauptfleisch in Pringsheim's *Jahrbücher*, Bd. 24, p. 173. Kienitz-Gerloff, *Botan. Zeitung*, 1893, holds the view that normally plasmic movement takes place in every living cell. This result, which merits careful attention, he arrives at partly from general considerations. If cells, removed from their normal connections, at first exhibit no movements, this, according to Kienitz, is the result of the mechanical injury. The matter requires further examination.

[2] See Berthold, *Studien über Protoplasmamechanik*, Leipzig, 1886.

[3] Further investigation of these matters is out of place here. See Bütschli, *Untersuchungen über Mikroskopische Schäume* (translated by E. A. Minchin); Verworn, *Bewegungen der lebenden Substanz*, 1892; Detmer, *Berichte der Deutschen botan. Gesellschaft*, Bd. 10, p. 436; Engelmann, *Ursprung der Muskelkraft*, 1893.

[4] See Velten, *Flora*, 1876.

[5] See Sachs, *Flora*, 1864, p. 67.

[6] See Pfeffer, *Zeitschrift f. wissenschl. Mikroskopie*, Bd. 7.

[7] See Sachs, *Textbook of Botany*.

[8] See also Clark, *Berichte der Deutschen botan. Gesellschaft*, Bd. 6, p. 273.

169. The Free Locomotion of Lower Organisms (Movements of Swarmers, etc.).

Many of the lower plants, like typical animals, are capable of free movement from place to place. The mechanism of these movements is still not clear, and so we shall not closely consider the matter. We shall, however, attempt to demonstrate the movements themselves, and determine how they are influenced by external conditions.

We will first take for examination Euglena viridis, which from the morphological point of view is certainly not related to typical algæ but to the infusoria, but which, in more than one physiological respect, is allied to the algæ. The material is easy to

obtain, being found in stagnant water, street gutters, etc. In order to obtain very vigorous individuals for examination, I first cultivate the Euglena material for a few days on bits of turf placed in a dish containing food solution such as used in water-culture experiments. The fragments of turf are only about half immersed in the fluid, and the green Euglena material is simply placed on their moist upper surface. The culture vessels are left for a few days before a window with a south aspect, and the fragments of turf are then placed in a porcelain dish, flooded with spring water, and left in the water for a few hours. During this time many Euglena swarmers collect in the water. Examination under the microscope shows that the body of Euglena viridis is spindle-shaped. Nucleus and chlorophyll bodies are present. At the anterior end, which bears a long cilium, are seen vacuoles and a red eye spot. According to Klebs the Euglena is clothed throughout its life by a membrane, and under favourable external conditions the Euglenæ pass into a resting state, in which they are immotile. The free forward movements of the Euglenæ are effected by means of the cilium, and are always accompanied by a rotation of the whole organism. In order to follow exactly these movements of progression, we dip a tube into the water containing the bits of turf, and introduce some of the swarmers into the hanging water drop of a small moist chamber, and observe under the microscope (as regards the preparation of the moist chamber, see 138). The swarmers are capable of movement both in complete darkness and in the light. The light nevertheless exerts a directive influence on the movement of Euglena, which therefore comes under the category of phototactic organisms. At the commencement of our experiment the swarmers are distributed pretty uniformly in the hanging drop of the moist chamber, but it can easily be determined under the microscope, that nearly all of them, especially if we cut off the light reflected from the mirror, very rapidly collect on the side of the drop towards the window, *i.e.* towards the source of light. If we rotate the slide, with the moist chamber, through an angle of 180°, the swarmers again fall into lively movement, and endeavour to reach once more the margin of the drop which is directed towards the source of light. We can, however, only observe these phenomena when the light to which the swarmers are exposed is not too intense. With more intense light, most of the swarmers collect, not at the illuminated edge of the drop, but at the

opposite edge. Under these conditions, therefore, they recede from the light.

If a shallow plate is filled with water well stocked with Euglenæ, and placed near a window, the swarmers collect, if the light is not too intense, on the window margin of the plate. If we rotate the plate through 180° most of the swarmers again rapidly group themselves on the window side. In such experiments with Euglena I have frequently observed such a lively movement of the swarmers towards the source of light, that the directive influence of the light rays could be demonstrated with the same material several times in the course of an hour.

Under certain conditions, especially when their free forward movement is arrested (*e.g.* in our moist chamber, by their collection in the illuminated margin of the drop), the shape of the Euglena swarmers changes in a striking manner (metaboly). Euglena viridis as a rule swells in the middle and draws itself out thin at the ends; the swarmers of other species of Euglena curve into the form of a crescent.

Suitable material for the observation of swarming movements is also found in Hæmatococcus lacustris, an alga which at Jena for example is found in the Leutra, colouring the stones on which it occurs a beautiful red. We place a few of the stones bearing the Hæmatococcus in a large shallow dish the bottom of which is only just covered with water, put a sheet of glass over the dish, and let it stand for several days. Then a number of the stones are placed in another vessel and flooded with water. We leave them in the water till the following day, and it will then usually be found that the water contains many red Hæmatococcus swarmers. Like those of Euglena, they are phototactic. If the light is not too intense, and the temperature is favourable (about 20° C.), they move towards the source of light. I have satisfied myself that to obtain large numbers of the swarmers, it is important to keep the stones for a few days, as above described, in a space saturated with moisture, before completely flooding them with water.[1]

Experiments similar to those which we have mentioned may be made with the small green swarmers of Clamydomonas. It is further to be observed that the swarmers in different stages of development may be tuned to light of quite different intensity, a fact which especially in the case of Hæmatococcus is not seldom very clearly perceptible. For example, when we study the

behaviour of the swarmers in the hanging drop we do not always find that in diffused light they seek the illuminated side. If the microscope is put close up to the window, they often collect on the side of the drop towards the room. The swarmers are then attuned to light of lower intensity, and in order to bring them to the illuminated edge of the drop, the microscope must be removed more or less from the window.

We make a circular hole 5–7 cm. in diameter in the window shutter of a dark room. In front of this opening may be placed glass flasks with parallel walls, containing either a solution of Potassium bichromate or an ammoniacal solution of Copper oxide The former solution is so prepared as to let through only the less refrangible rays (red, orange, yellow, and some green), the latter absorbs these rays and transmits only the more refrangible rays. If a dark room is not available a large box may be used, the inside of which is covered with dull black paper. We now expose swarmers in the hanging drop to light of different wave lengths. Under the influence of the less refrangible rays the organisms behave as in darkness; the rays transmitted by the ammoniacal Copper oxide solution however, like mixed white light, materially influence the direction of motion of the swarmers.

For the method to be employed in investigating the behaviour of the swarmers in the individual groups of rays in the objective spectrum, see Strasburger's cited work; also Pfeffer in *Botan. Zeitung*, 1872, No. 23. Here and in many other cases the spectrum obtained by means of Rauland's grating might be recommended for investigations in plant physiology.

Even under the influence of gas-light the swarmers exhibit their phototactic peculiarities. If between the gas-light and the microscope we interpose in a suitable manner a concentrated solution of alum, the organisms react in the usual way to the stimulus of the light. Their reaction is, however, arrested when a solution of Iodine in Carbon disulphide is interposed. Since the alum solution is highly athermanous, while the Iodine solution is strongly diathermanous, we may conclude from the above experiment that dark heat rays are without influence on the direction of movement of the swarmers.

Also the swarmers of algæ, as can easily be determined by employing strips of paper according to the method described in 171, possess no rheotropic properties. But these swarmers exhibit geotropic irritability, as the following experiment seems

to indicate.[2] We suck up some water containing Euglenæ into a capillary tube 0·5 mm. in diameter, open above and below. If we place the capillary in a vertical position in the dark, the swarmers collect in its upper region.

It is of interest that the swarmers of many algæ are distinctly aerotropic. This has been specially demonstrated in the case of Euglena by Aderhold, and we make the following experiment to acquaint ourselves with the phenomenon. A small dish is filled with water containing large numbers of Euglena swarmers. We also fill a test-tube to the brim with the water, close it with the thumb, and invert it with its mouth under the water in the dish. Light being excluded, it will be found after some time that almost all the swarmers have left the water in the test-tube, and collected in the water in the dish. This is by no means due to positive geotropism or other causes, but is explained by the aerotropism of the swarmers. They strive to reach places in which larger quantities of Oxygen are available. This is very clearly brought out by the following observations. When the swarmers have left the test-tube, we replace some of its water by air, spread a layer of oil on the water in the dish, and place the whole in the dark. The swarmers now pass in considerable numbers from the dish into the test-tube, because here larger quantities of Oxygen are presented to them.

The organisms known as Oscillariæ have a wide distribution in stagnant waters and on muddy soil. They are for the most part blue-green in colour, and occur in the form of filaments. These organisms exhibit various movements which can be closely followed under the microscope. Especially striking are the irregular curvatures which the filaments undergo as they move forwards or backwards.

In the cases hitherto mentioned the movements have been due to the activity of the organisms themselves, but there are still to be mentioned a number of curious phenomena which are due to purely passive movements on the part of swarm spores.

If green-coloured water, containing large numbers of swarming Clamydomonads or Euglenæ, is poured on to a plate, which is then covered with a sheet of glass and placed under a cardboard box in the middle of a large room, we shall find after a time that the algæ have grouped themselves in the form of concentrically arranged clouds or so as to form other regular figures. If we remove the sheet of glass covering the plate, the figures rapidly

disappear. If a plate into which alga-containing water has been poured is so placed (*e.g.* in front of a window) that one side becomes somewhat warmer than the other, it will be found (even when the light is excluded) that the swarmers collect according to circumstances on one or the other side of the plate. According to Sachs [3] all these phenomena are caused by currents in the water, which group the swarmers in a definite manner, and are themselves dependent on the distribution of temperature. Sachs was led to this conclusion by the study of emulsion figures. To investigate emulsion figures, we pour pure olive oil over coarsely ground alkanna root, and after twenty-four hours filter off the intensely red coloured oil resulting. We further make in a glass cylinder a mixture of water and alcohol, whose specific gravity is exactly 0·920. This fluid has almost exactly the same specific gravity as olive oil. If we pour a small quantity of it into a beaker, and then add some of the red olive oil extract, large oil drops will rise very slowly in the fluid, which is therefore of somewhat higher specific gravity than the oil.

These preliminaries having been arranged, we add 5 c.c. of the red oil to every 500 c.c. of the alcohol mixture, and shake violently, so as to break up the large oil drops into thousands of minute droplets, and in this way the necessary emulsion fluid is prepared.

For use we pour it into a flat porcelain dish to a depth of about 10–15 mm. We cover the dish with a sheet of glass, or leave it uncovered, and observe how the moving oil drops at first form series of dots and networks, and after a time (one-quarter to half an hour) group themselves into regular figures. If we have covered the dish with a sheet of glass after pouring into it the emulsion fluid, and now remove it after the formation of the figures, they rapidly dissipate under the eye of the observer.

The form of the emulsion figures is very varied. In Fig. 139 (*B*) is indicated one which frequently occurs. Such concentric figures, however, only form when the dish containing the emulsion fluid is placed in the middle of the room. Polar figures, such as the one indicated in Fig. 139 (*A*), form when we place the fluid near a window or a stove, so that one side of the dish is warmer than the other. If we experiment, *e.g.*, with the emulsion described, in which the coloured oil is only very little lighter specifically than the mixture of alcohol and water, the pole and marginal lines of the polar figure are always directed towards the colder side of the

dish. Emulsion figures are to be referred to currents in the fluid, dependent themselves on temperature relations. The emulsion figures have a very great similarity to the figures in which zoospores arrange themselves under the conditions described above, and the same causal forces underlie all these phenomena.

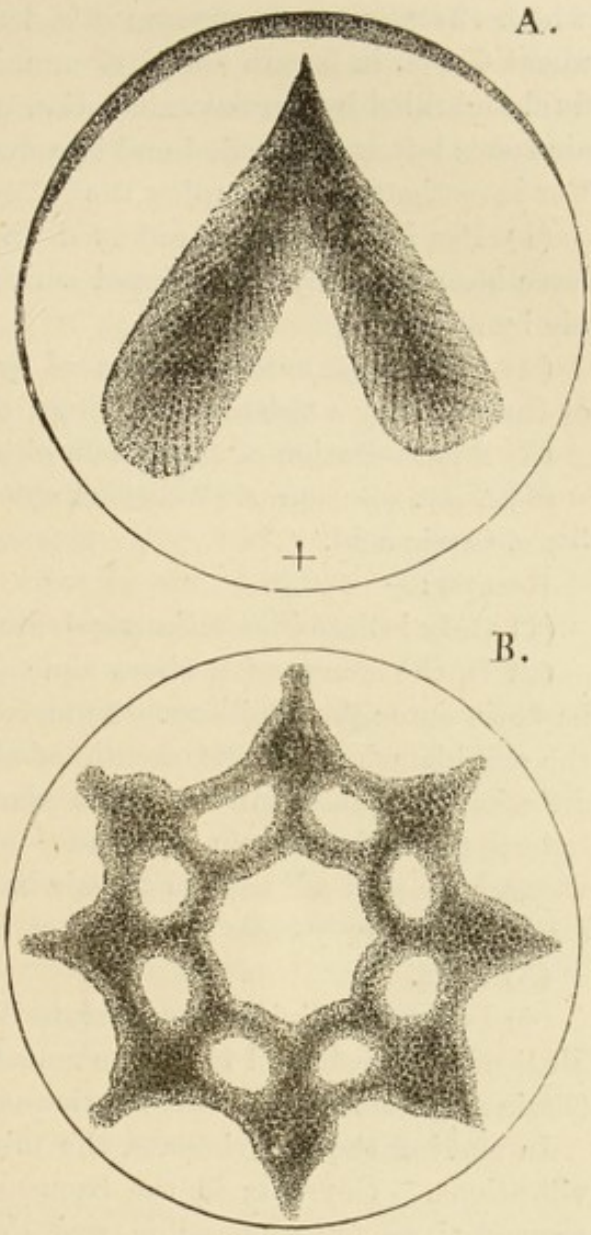

FIG. 139.—Emulsion figures. (After Sachs.)

It is also of considerable interest to study the influence of various substances on the movement of bacteria swarmers. The aerotactic behaviour of these swarmers has already been indicated in 11. We will now investigate their so-called chemotactic movements.*[4]

We kill a pea see by immersion in boiling water, put it into 100 c.c. of water, and let it stand for a day or two, till large numbers of Bacterium termo have developed in the fluid. The fluid is filtered through coarse paper, to remove the larger aggregations of bacteria, and at once employed for the observations.

Under higher powers we see that the swarmers of Bacterium termo move slowly or rapidly, sometimes forwards, sometimes backwards. We now apply to the bacterium-containing drop, lying on the bare slide, a capillary tube containing the solution

* Of course the movement of the organisms to places relatively rich in Oxygen is itself only a particular example of chemotaxis.

whose effect on the bacterium it is desired to test. The capillary, about 6 mm. in length and 0·06 mm. in diameter, is put into the fluid and filled by partial exhaustion under the air pump till the air space left at the sealed end is reduced to a length of say 3 mm. For investigation we employ the following fluids :—

(1) Pea infusion prepared as described, in which the bacteria have been killed by boiling, and which has been re-saturated with air by shaking ;

(2) 1 p.c. meat extract, prepared by dissolving 0·5 gr. of extract of the ordinary consistency in 50 gr. of water ;

(3) 2 p.c. solution of Potassium chloride ;

(4) 2 p.c. solution of Potassium chloride to which has been added 5 p.c. Citric acid.

Results :—

(1) Only isolated bacteria penetrate into the capillaries.

(2) In the course of a short time (a few minutes) very many bacteria enter the capillary. Numerous swarmers also collect in the neighbourhood of the mouth of the capillary. Gradually, as diffusion from the capillary takes place, these latter bacteria distribute themselves again, and those in the tube, owing to want of Oxygen, in part advance to the air bubble, here gradually forming a second and very close aggregation.

(3) As 2.

(4) Isolated individuals penetrate into the capillary, and perish. Most of them rebound from the mouth of the capillary, since the Citric acid exerts a repulsive action on them.

In making the experiments, the microscope must be free from vibrations. Currents in the bacteria-containing drop are to be prevented as far as possible, and the capillaries containing the fluids under investigation must, before being pushed up to the drop containing the swarmers, be rinsed on the outside by being rapidly passed through water.

In order to prove that lower organisms are galvanotropic, it is very convenient to work with Paramecium, *e.g.* Paramecium aurelia.[5] This infusorian, elongated in form, contains a nucleus and two alternately pulsating vacuoles, and is provided with numerous cilia. If a handful of hay is treated in a large vessel with pond water, then in the course of eight to fourteen days there develop in the fluid, besides bacteria, so many Paramecia that it appears milky.

We now put together the following apparatus. The wires

from a galvanic battery* of several elements are connected with a current reverser (commutator). From the commutator pass two other wires, the ends of which are attached to unpolarisable electrodes (see 63). In the course of one of these wires is intercalated a key.

We now set up a microscope. On the stage is placed a watch-glass charged with Paramecium-containing fluid. Even under low powers we see the organisms in active movement, crossing the fluid in all directions. If we introduce the electrodes into the fluid and close the circuit, all the Paramecia, as if by command, direct their anterior end towards the negative electrode and move towards it. In a short time the fluid in the neighbourhood of the anode is quite devoid of Paramecia; they have all collected at the kathode. If we break the circuit, the infusoria direct their anterior end towards the anode; they move towards it and collect there, but the aggregation is not so complete as at the kathode on making the circuit, since the organisms soon distribute themselves again uniformly throughout the fluid.

The Paramecia having collected at the kathode on completion of circuit, and the commutator being now reversed, the organisms at once execute movements corresponding with the new conditions, and they can be forced to turn round every moment by reversing the commutator. In Euglena viridis no galvanotropic peculiarities have so far been determined with certainty.

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[1] See Strasburger, *Wirkung der Wärme und des Lichts auf Schwärmsporen*, Jena, 1878, and Klebs, *Untersuchungen aus dem botan. Inst. zu Tübingen*, Bd. 1, H. 2.

[2] See Aderhold, *Jenaische Zeitschrift f. Naturwissenschaft*, Bd. 22, and Jensen, *Archiv. f. d. ges. Physiologie*, Bd. 53.

[3] See Sachs, *Flora*, 1876.

[4] See details in Pfeffer, *Untersuchungen aus dem botan. Inst. zu Tübingen*, Bd. 1, p. 450, and Bd. 2, p. 584.

[5] See Verworn, *Pflüger's Archiv f. d. ges. Physiologie*, Bd. 45 and Bd. 46.

170. Movements of Chlorophyll Bodies.

The movements of the chlorophyll grains in plant-cells are of great biological importance. We have here to do, as it would appear, not with movements due to the chlorophyll grains them-

* The current with which we work must be neither too weak nor too strong. Strong currents kill the infusoria.

selves; the changes in position which they undergo are caused by movements of the cell protoplasm. To gain exact information respecting the movements of the chlorophyll bodies, it is advisable to select for examination moss leaves or fern prothallia, for in the palisade parenchyma of ordinary foliage leaves the chlorophyll grains usually experience only inconsiderable changes in position, and in the spongy parenchyma, in whose cells it is true considerable changes in the position of the chlorophyll grains may take place, the relations in question are often, for various reasons, not easy to follow.

If plants of Funaria hygrometrica or fern prothallia (found without much difficulty in greenhouses in which ferns are grown) are kept for some time in darkness, under otherwise normal conditions, the chlorophyll bodies pass into the darkness position, *i.e.* in the case before us they travel to the cell-walls running perpendicularly to the surface of the object. In other cases, however, the chlorophyll bodies do not behave in exactly the same manner. If the shading has not been continued too long (only for several hours), so that the contents of the cells have not gone over into a condition of darkness-rigor, but are still phototonic, it will be found that the chlorophyll bodies are peculiarly sensitive to light. Sods of Funaria or fern prothallia, which have been kept in darkness, are exposed to diffused light in such a way that the plants receive the rays of light from above. After a few hours we place a few Funaria leaves, or a few prothallia, in a drop of water on the slide, lay on a cover-glass and observe. The chlorophyll bodies are no longer disposed on the side walls of the cells, as in the darkness position, but have arranged themselves on the front and back walls, and moreover turn themselves broadside to the observer (surface position). We now expose our preparations, on the slide, to direct sunlight, keeping the outside of the cover-glass well moistened with water, so as to avoid overheating. If we examine after a few minutes, we shall perceive that the chlorophyll bodies are still in the same position as before, but their form has changed. The previously polygonal grains have drawn in their angles; they have become rounded, and the chlorophyll bodies are clearly striving to present as small a surface as possible to the too intense light. This rounding off of the green constituents of the cells is due to movement of the chlorophyll bodies themselves; but beside this, exposure to very intense light induces a change in the position of the chlorophyll bodies, which is brought about by the proto-

plasm. If, viz., we expose our preparations to direct sunlight for a considerable time (in my experiments with Funaria leaves for $\frac{3}{4}$ hour) the chlorophyll grains will pass from the outer and inner walls of the cells to the side walls, and dispose themselves in the profile position. The grouping of the chlorophyll grains in the surface position on the outer and inner cell-walls is, however, resumed if the preparations are exposed for some time to diffuse daylight. There is no doubt that these changes in position of the chlorophyll bodies are of biological importance to the plants. In diffuse light the green constituents of the cells take up a position which will enable them to make use of the rays of light for the purpose of assimilation in a very thorough manner, while they will be protected from the destructive influence of too intense light and too great warmth by the arrangement on the side walls of the cells.[1]

The movements of the chlorophyll bodies caused by the stimulus of light are only exhibited when the cells are exposed to normal conditions of life. Too great reduction of temperature, insufficient moisture, and want of Oxygen prevent them, as can easily be proved by suitable experiments.

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[1] See Stahl, *Botan. Zeitung*, 1880, p. 321.

171. The Movements of the Plasmodia of Aethalium septicum.

The plasmodia of the Myxomycetes are capable of peculiar movements; they can creep from one place to another, their outlines constantly changing in the process. These movements are very largely influenced by external conditions, a fact which is of the utmost biological importance to the plasmodia. Aethalium septicum is chiefly found on tan. The yellow plasmodia are to be found in spring (*e.g.* in May, during which month I made numerous experiments on the plasmodia) on the older masses of tan. There further appear, however, on the tan, at this time, as also in winter, Aethalium sclerotia, in the form of small knob-like yellow masses, about 2 mm. in length, from which we can readily obtain plasmodia. It must here be emphatically remarked, that the plasmodia are very delicate structures, easily killed, and sensitive to contact with the hand. Consequently the masses of tan, with the plasmodia traversing them, must be handled care-

fully, and it is best to convey the tan from the tannery to the laboratory in a box, without fingering it much. We now conduct the following experiment :—

A small strip of Swedish filter-paper is moistened and dipped at one end into a beaker half full of water. The other end of it hangs freely downwards over the edge of the beaker, and during the experiment is spread out on the plasmodium-bearing tan. If we now place the whole preparation in darkness, in a room whose temperature is 25° to 30° C., plasmodia soon leave the tan, and creep further and further up the strip of filter-paper. This migration of the plasmodia, on a substratum thoroughly saturated with water, is not due to hydrotropism, but is induced by currents of water. The plasmodia are therefore rheotropic, and in fact they always travel against the current of water.

The plasmodia, however, react also to variations in the distribution of water in the substratum ; they are not only rheotropic, but also hydrotropic. To prove this we convey plasmodia, which have collected on blotting-paper under the influence of a current of water, to the middle of a sheet of glass covered with several folds of moist filter paper. The plasmodia, when placed in a dark chamber in which the air is saturated with moisture, spread out uniformly on the horizontal and water-saturated substratum. If the research material is now placed in a dark chamber which however is dry, and we fix at a short distance above the plasmodia an object-glass smeared with dilute gelatin, an interesting appearance is soon (often after a few hours) to be observed. The blotting-paper gradually dries, and the plasmodia withdraw from the drying portions of the substratum, but accumulate under the moisture-distributing object-glass. The plasmodia thus exhibit positive hydrotropism. It must be remarked that our plasmodia, during the greater part of their development, react as above to variations in the distribution of moisture. Plasmodia near the time of sporangium development are, on the contrary, negatively hydrotropic.

The plasmodia of the Myxomycetes are not at all geotropic. This can be demonstrated by placing strips of blotting-paper with plasmodia against a vertical, moist substratum (*e.g.* paper saturated with water, and lying on a sheet of glass) in a dark chamber saturated with moisture. Under these circumstances the plasmodia spread out uniformly in all directions on the substratum.

Light of any great intensity, as it appears, *e.g.*, diffuse daylight, plasmodia always avoid. If we expose pieces of tan traversed by plasmodia to the light, in the moisture-saturated chamber, the plasmodia retract into the tan. Similarly plasmodia spread out on sheets of glass, when exposed to the light, seek places where the intensity of the light is least, *i.e.* shaded places. The plasmodia are easily transferred to sheets of glass, even object-glasses for example, as follows:—A beaker is filled with water, into which is brought one end of a strip of blotting-paper whose width is somewhat less than that of our object-glass. The other end of the blotting-paper is laid on the face of an object-glass standing vertically beside the beaker. The whole arrangement is placed on a layer of sand, which takes up the water running down from the object-glass. At the base of the object-glass is next put a piece of tan bearing a plasmodium, the tan being of course leaned against that face of the glass down which the water is running. We finally cover with a bell-glass, and exclude the light. The plasmodium, in virtue of its rheotropism, now creeps on to the object-glass, and spreads out on it. If for the sake of closer examination we desire to have plasmodia on the slide under a cover-glass, it is a good plan to fix the cover-glass on the side of the slide down which the current of water is flowing, by means of bits of wax placed under the four corners. It is then possible for branches of the plasmodia to creep under the cover-glass, and here spread out forming delicate threads. The accumulation of the plasmodia on the slides does not always proceed with the same rapidity; generally it is sure to take place in the course of half a day.

It is of very special interest to study exactly the attractions and repulsions exerted by different substances on the direction of movement of plasmodia. We employ for the experiments plasmodia coaxed on to blotting-paper by means of a current of water, or the necessary material is obtained from Aethalium sclerotia, by putting them on a damp surface (several layers of blotting-paper saturated with water). I obtained in this last way beautiful plasmodia, and when they had been made fairly hungry by being kept for a considerable time under a bell-glass, a condition favourable for the experiment, small balls of blotting-paper saturated with tan extract were laid on them. The substances present in the tan extract exert an attractive influence on the plasmodia, and hence, even after a few hours, the paper balls

are seen to be traversed in every direction by plasmodial strands. The trophotropism of the fungus is thus established.

About the middle of a plasmodium, spread out on a moist substratum, we place a small crystal of common salt. The part of the fungus in direct contact with the salt becomes brown and dies, while those portions of it which are not killed retract from the salt, so that gaps are formed in the plasmodium, which however may close up again if the gradually dissolving salt becomes uniformly enough distributed in the moist substratum. Sodium chloride therefore exerts not an attractive, but a repulsive action on the plasmodium.[1]

That the extraordinary sensitiveness of the plasmodium to the influences above mentioned, as also to others, is of biological importance to their delicate organisation will be clear from what has gone before, and we need not here consider the matter further.

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[1] See Stahl, *Botan. Zeitung*, 1884, No. 10. Here also will be found instructions for further experiments, together with citations of literature.

II. GEOTROPIC, HELIOTROPIC, AND HYDROTROPIC NUTATIONS, AND SOME OTHER PHENOMENA OF IRRITABILITY.

172. The Geotropic Behaviour of Roots.

The roots, especially the main roots of plants, strive to grow vertically downwards, a phenomenon which is due to the positive geotropism of the organs. To prove that roots are positively geotropic, we conduct the following experiment:—Seeds of Pisum, Vicia Faba, or Phaseolus are soaked for twenty-four hours in spring water, and then placed in large flower-pots or wooden boxes containing moist sawdust. The sawdust must be very loose and uniformly moistened throughout in order to ensure the normal development of the seedlings. The seeds of Vicia Faba are laid in the sawdust with their micropyles downwards, so that the emerging main root need make no curvature. The seeds of Phaseolus we lay horizontally; the main root then makes a right angle with the long axis of the seed on emergence. We

cover the flower-pots or boxes, as the case may be, with large cardboard boxes, or put them in a cupboard, and when the roots have reached a length of about 3 cm. we take out of the sawdust a few seedlings with very straight roots. After careful washing we pass long pins through the seedlings and fix them in the manner indicated in Fig. 140, the roots being directed horizontally, and the whole being covered by a sufficiently large bell-glass. In transfixing the seedlings attention must be paid to what was said on p. 390. The rim of the bell-glass dips into water, contained in a glass dish, while the inside of the glass is provided with wet blotting-paper. We place our apparatus in darkness. We may also make the experiment by removing some of the seedlings from the sawdust and simply replacing them with their roots directed horizontally. At a sufficiently high temperature (20° C.–25° C.) we can, even after a few hours (at a low temperature not till later), perceive a downward curvature of the root-tip, more or less sharp according to circumstances. The root executes a positive geotropic curvature, and continues to grow not horizontally but downwards. If seedlings of Phaseolus, etc., are laid in sawdust with their roots directed vertically upwards, the tip of the root soon curves downwards, and then continues to grow in that direction. In my investigations with Phaseolus (at 22° C.) the root-tip had already made a considerable curvature at the end of four hours. If seedlings are laid in sawdust with their roots directed obliquely upwards or downwards, geotropic curvatures naturally are made which tend to carry the root ends vertically downwards.

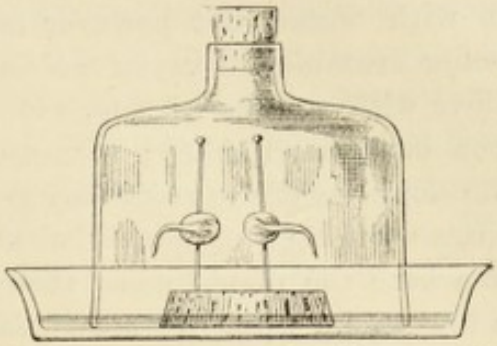

Fig. 140.—Apparatus for demonstrating geotropic root curvatures.

For further more searching investigations relating to the geotropic downward curvature of roots, we require first of all a special box, such as the one drawn in Fig. 141, and first employed by Sachs. The box consists mainly of strong sheet zinc. The front and back, however, are formed by sheets of glass about 20 cm. high and 30 cm. broad. These must not be perpendicular, but inclined at an angle of about 10°. The bottom of the box

and its ends are pierced with numerous small holes, to promote the ventilation of the soil contained in the box. We use light, very humous soil such as is employed for greenhouse plants, moisten it with water, not however adding too much to prevent its being crumbled between the hands, and then pass it through a sieve with meshes 1·5 mm. wide. In filling the box the soil must not be pressed down; it must be loosely packed, so that the development of the roots may go on smoothly. The requisite seedlings have been cultivated, as above described, in moist sawdust. We take the plants when their roots have attained a length of a few mm. First we must make fine ink lines on the roots to serve as marks. We dry the roots carefully with a piece of linen,

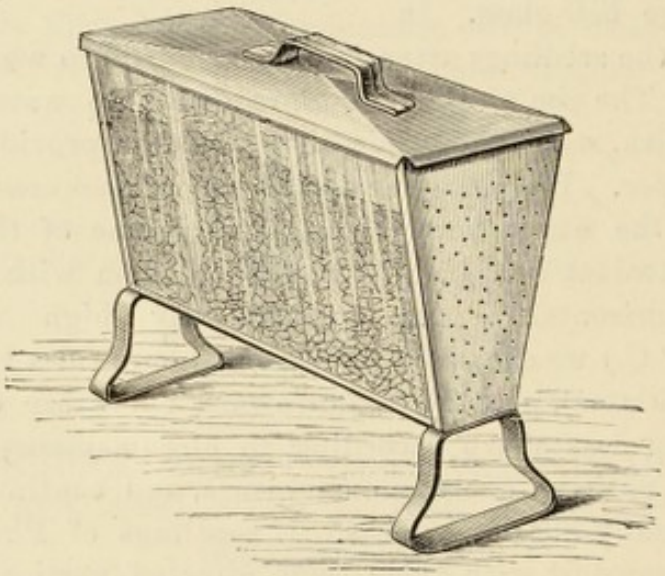

Fig. 141.—Zinc case with glass walls for observations on root development.

and then make the ink-marks, at intervals of 2 mm., with a sable pencil. This operation, which must be carefully performed, is best managed with the help of a large smooth sheet of cork about 2 cm. thick, on the left side of which a number of large notches of different sizes are made with a round file, from which, along the surface of the cork, pass grooves made with a thin round file. In the notches are placed the seeds; the grooves receive the roots, and alongside them is placed a millimetre scale. The seedlings when ready are placed in the soil of our box, with their roots directed horizontally and closely applied to one of the glass faces, loosely covered with soil, and observed. We fasten on the outside off the glass a small triangular piece of paper, of

which one angle accurately points to the first mark placed just behind the root tip. In Phaseolus I found, as Sachs mentions for Vicia also, that the root for about an hour continued to grow horizontally; the first mark becomes somewhat displaced from the point of the paper. After that time, however, a geotropic curvature of the root soon became noticeable, and observations repeated from time to time showed that the curvature can first clearly be proved in the zone lying between the first and second marks; then later it occurs in the succeeding zones also. We determine that all the zones of the root undergoing growth in length take part in bringing about the geotropic downward curvature; but in order to confirm this important observation we make the following experiment. We use, for example, seedlings of Vicia Faba with roots 2 cm. long. After the growing region of the root has been divided by ink-marks into zones of 2 mm. length, the seedlings are laid in the culture box in the manner above indicated. After about eight hours at 20° C. the horizontally placed root has already made a conspicuous curvature, and we can proceed to measure the growth which has taken place. We use for the purpose a thin sheet of mica on which a number of concentric circles are scratched with a pair of compasses.* It is now best to subdivide the quadrants not into 90 parts, but by easily and accurately performed successive halving into 8, 16, or 32 parts. We calculate the length of the arc for each circle. The tabulated values serve for the determination of the lengths of the arcs of the curved roots. The mica sheet is laid against the glass pane behind which the root is situated. We find which circle agrees with the curvature of the convex side of the root, or with a portion of it, fix the mica to the glass by means of strips of gummed paper, and make the further observations. In our case the total growth on the convex side of the root amounts to about 4 mm. The entire growing region of the root has participated in bringing about the geotropic downward curvature. The most rapid growth has taken place in the third zone. We further seek pairs of seedlings of Vicia Faba very uniform in development, which have been grown in moist sawdust, and with roots from 1·5 to 2 cm. in length. From the growing point onwards at intervals of 2 mm. we make fine ink-lines as marks (the first being at the growing point itself), and lay one of the seedlings in the

* The largest circle may have a radius of 20 mm.

soil of the box with its root directed perfectly horizontally along the glass, while its fellow is placed with its root pointing vertically downwards. After twelve to sixteen hours (20° C.), we measure the growth in the straight and geotropically curved roots respectively, in the latter case making use of the sheet of mica, and determining not only the growth of the convex, but also that of the concave (lower) side of the root. If, *e.g.*, the growth on the convex side of the root = 10 mm., that on the concave side = 6 mm., the growth along the median line (axis of the root) amounts to 8 mm. The straight, control root may have lengthened by 9·5 mm. Thence it follows, as Sachs first made clear, that the convex side of curving roots grows somewhat more rapidly than a straight root, under similar conditions. The concave side of the curved root grows considerably more slowly than the straight root, and the total growth of the former is notably less than that of the latter.

In researches on the growth of the main roots of Phaseolus or Vicia Faba, we should not omit to make ourselves acquainted in a general way with the form of the geotropic curvature which the roots have experienced. For this purpose we provide ourselves with the thin sheet of mica on which a series of concentric circles has been scratched with compasses. By laying the sheet of mica over the glass surface against which the root is growing, we can readily determine the form of the root curve. At the commencement of the geotropic nutation, it coincides with a circle of considerable radius. Later the root curve appears less flat than in the first stage of observation. Later still the curve no longer coincides with any of the circles, but becomes parabolic. The zone of most active growth is strongly curved; in front of this region and behind it the curve is much flatter.

We will now proceed to consider the geotropic behaviour of secondary roots, confining ourselves to the lateral roots of the first and second order given off by the main roots of Phaseolus, Pisum, Vicia, and Zea. Seedlings are cultivated in our culture case behind a sheet of glass. The main root grows straight downwards. The lateral roots of the first order, forming in acropetal succession on the main root, do not take this direction, but grow, as is indicated in Fig. 142, more or less obliquely downwards. We can easily satisfy ourselves that the lateral roots of the first order do actually behave geotropically, by inverting the case, so that the main root is directed upwards. We

then find after some time that the ends of the advancing secondary roots have turned downwards in a curve. The secondary roots of the first order, therefore, in contrast to the main roots, do not grow vertically downwards; their positively geotropic downward curvature ceases when they form a certain angle with the vertical, the geotropic "limiting angle." It may further be noted that the lateral roots of the second order, springing from the lateral roots of the first order, are not at all geotropic; they do not react to the influence of gravity.[1]

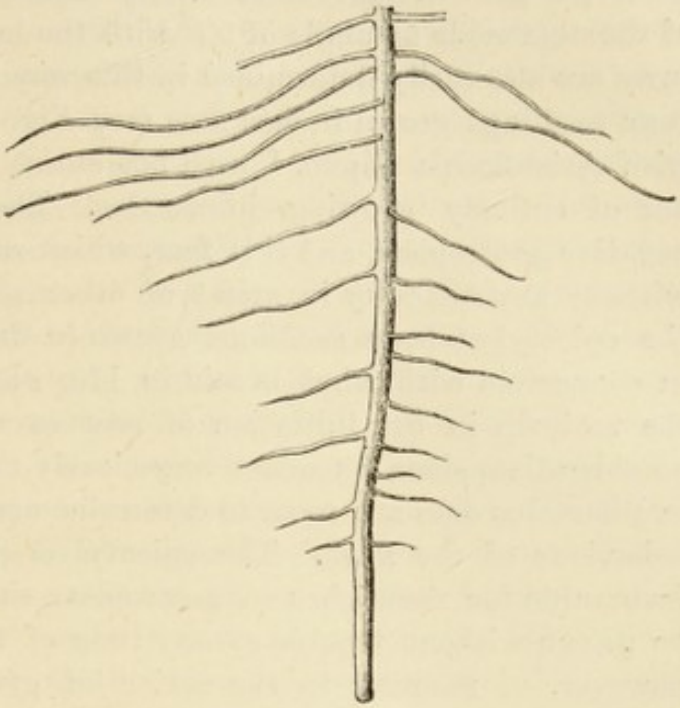

FIG. 142.—Portion of a root of Phaseolus multiflorus growing behind a sheet of glass.

[1] The literature concerning the geotropism of roots is collected together in my *Lehrbuch der Pflanzenphysiologie*. As regards the method of investigation, consult Sachs in *Arbeiten d. bot. Inst. in Würzburg*, Bd. 1.

173. The Geotropic Behaviour of Shoots.

In many stems negative geotropism is extremely well marked, and we will investigate these in order to acquire a further insight into the influence of gravitation on plants. If negatively geotropic organs are laid horizontally, they curve upwards, as can easily be demonstrated in a large variety of cases. We cover the bottom of a large zinc box with moist sand, heap the sand fairly high against the walls, and stick into the slopes the lower end of the piece of stem whose geotropism is to be investigated, the rest of the stem being free. We then close the box with a cover. When, *e.g.*, shoots of Chrysanthemum Leucanthemum bearing

flower buds were placed horizontally in the moist atmosphere of the box, in darkness, I found that, at a temperature of 24° C., they had even after a few hours curved strongly upwards. The geotropic growth movement finally ceased when the upper part of the stem made an angle of 90° with the lower. Geotropic curvatures are also easily determined in this way in severed epicotyls of bean seedlings grown in darkness (see Fig. 143). In experiments with Aristolochia Sipho, I used not entire shoots but pieces cut out of actively growing internodes. They exhibited vigorous negative geotropism, and this fact, which can also be determined without any difficulty in stems of other plants (*e.g.* portions of the epicotyl of bean seedlings grown in darkness), is of interest in connection with what is said in 175, where the significance of the root tip in the initiation of root curvatures will be under consideration, since it teaches very clearly that the tip of the stem in Phaseolus does not seem to determine exclusively the geotropic behaviour of the stem. The epicotyl of Phaseolus is specially favourable for researches on geotropism, since bean seedlings can be grown without trouble at any time of the year. The power, however, of reacting to the action of gravity is by no means quantitatively the same in different plant structures. While the

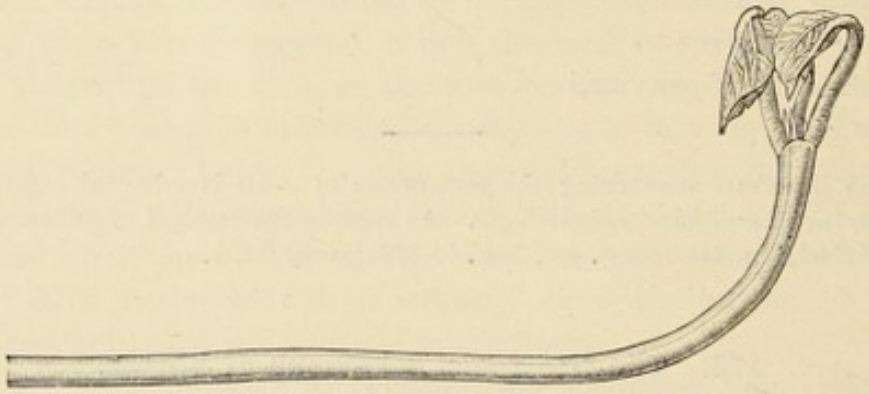

Fig. 143.—Epicotyl of Phaseolus multiflorus, exhibiting a negative geotropic curvature.

structures above mentioned curve very quickly and vigorously when they have been placed in a horizontal position in a moist place, young defoliated shoots of Sambucus nigra, *e.g.*, under similar conditions only slowly execute geotropic curvatures. The plumule of Triticum vulgare possesses great geotropic irritability. If small pots in which wheat seedlings with plumules about 2 cm. long have developed, are laid horizontally, the plumules rapidly curve upwards.

The following experiment, which I frequently repeated, is instructive. We stick the lower end of a leafy shoot of Hippuris vulgaris into the moist sand of our zinc box. When the shoot has been kept in a horizontal position for one to one and a half hours at a high temperature, a distinct though still not very strong curvature is already perceptible. We now place the shoot in a vertical position with its lower end in moist sand, cover with a bell-glass, and screen from the light. To our astonishment we observe after a time that the curvature initiated when the stem was in a horizontal position becomes greatly intensified now that it is in a vertical position. We have here a phenomenon depending on geotropic after-effect, and therefore also soon replaced by another. The curvature, viz., which at first became more marked when the stem was placed in a vertical position, gradually disappears completely; the shoot directs itself straight upwards, since, after the geotropic after-effect has passed off, gravity acts in the usual manner on its curved end. The phenomenon of geotropic after-effect can be made out also with other shoots.

In most plants, the power of curving under the influence of gravitation is confined to the terminal internodes. The fully grown parts of the shoot, lying further back, no longer react to the action of gravity. So much the more remarkable, therefore, is the geotropic behaviour of the haulms of grasses. Between the successive, sharply differentiated internodes, are situated, as is well known, the nodes, which are easily recognised by their colour and swollen form. These are nothing but the basal portions of the leaf-sheaths. While the parts of the haulm lying between them may already have become mature, rigid, and hard, these nodes retain their youthful character for a comparatively long time, and hence also are easily able to effect geotropic growth curvatures. This power of growth, it is true, finally dies away in the cells of the nodes also. The younger nodes of grass haulms are capable of more vigorous geotropic curvatures than the older ones, since their parenchyma still turgesces very energetically, and since their cells are still capable of very active growth. If, *e.g.*, we cut out from the haulm of a flowering rye or barley plant, a number of pieces each about 10 cm. long, and each with a node at its middle, and then at once dispose them horizontally in our zinc box, we shall find that the younger pieces, say at the end of twenty-four hours, have curved much more vigorously than the older ones. By measuring the angle, the extent of the curvature

may be accurately specified. The oldest pieces of the haulm no longer curve at all. The form assumed by grass haulms which have raised themselves geotropically, is shown in Fig. 144. We can easily satisfy ourselves by experiment that not only intact haulms, but also haulms longitudinally split are capable of curving geotropically.

If some sections of grass haulms, each with a node at its centre (I experimented with Hordeum), are stuck horizontally into moist sand, and others of the same age obliquely upwards, we find after one to two days that the former have curved more vigorously than the latter, as is at once shown by measuring the angles. From this experiment, which may be repeated with any stems which react energetically to the stimulus of gravity, it follows that the effect of gravitation on plants is greater, the more nearly the angle at which it acts approaches a right angle.

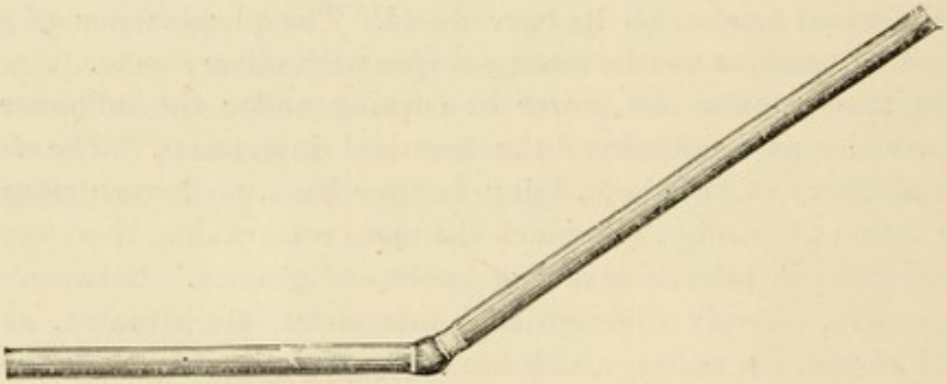

Fig. 144.—Piece of a grass haulm, curved geotropically.

Finally we will make a number of experiments on the growth of geotropically curving grass nodes and other plant structures. It is well known that under normal conditions the nodes, after attaining a certain stage of development, cease to grow. Strangely, however, growth re-commences in the cells of the node, when the grass haulms are placed in a horizontal position.

We cut out lengths of rye or barley haulms, each with a node at its middle, mark the length of the nodes on two sides by means of fine ink-lines, and place the objects in our zinc box. After two or three days we again measure the distance between the ink lines on the geotropically curved pieces of haulm, using a strip of paper divided into millimetres. It is seen that the convex lower side of the node has increased considerably in length, while the concave upper side has become shorter owing to the compression of its

tissue. Thus when negative geotropic curvatures take place the energy of growth of the cells, at any rate of the lower side, which becomes convex, is very considerably increased.

It is very instructive to undertake the following investigation, which I made with specially good results using pieces of oat haulms which had undergone vigorous geotropic curvature. We prepare radial longitudinal sections of the node, and examine them under the microscope. The cells of the parenchyma of the underside are at once seen to be considerably elongated in the direction of the long axis of the organ, while the cells of the upper side are tabular in form. They are shorter in the longitudinal than in the radial direction. The convexity of the lower side of the geotropically curving grass nodes is due, therefore, to vigorous stimulation of the growth of its cells.

For further observations on the growth of geotropically curving plant structures, stems of Sida Napæa or Inula Helenium are specially suitable. We use shoots deprived of their leaves and end buds, composed of a few internodes, and 200–300 mm. in length. They must be perfectly straight and require to be carefully selected. We take nine such pieces, cut them all to the same length, and arrange them in groups of three. Those of the first group we at once analyse by cutting off with a sharp razor two strips of the cortex and determining their length. Three other pieces we bring into our zinc box containing moist sand, and place them horizontally. The last three we put in a slightly inclined position into a glass cylinder, the bottom of which is covered with moist sand and which can be closed with a cork. After twenty-four hours the second and third group are analysed. We remove strips of cortex from their concave and convex sides respectively, and measure their length. If now we compare the average numbers in the second and third groups, it stands out that, in the former, in which the stems were laid horizontally and therefore curved vigorously, the growth of the now convex side must have been greater, that of the now concave side less, than that of the corresponding sides of the stems placed in the cylinder, and hence not at all or only slightly curved.[1]

If we experiment on shoots with apical growth (*e.g.* epicotyls, of Phaseolus), laying the shoot horizontally in the zinc box, after marking it with fine ink-lines in the manner given on p. 385, in order to determine the growth in the successive zones, then at a particular time (after about twelve hours) we find the strongest

curvature in the most actively growing region of the stem, but later when the geotropic erection has come to an end, the greatest curvature is at the base of the growing region, since here gravitation continues to act for a time as a stimulus. If we experiment with very rapidly growing thin shoots (*e.g.* Agrostemma) it is found that at a certain time, the geotropic nutation of the entire shoot however having as yet by no means come to an end, the summit of the shoot is not vertically upright, but owing to geotropic after-effect (see p. 443) is carried far beyond the vertical. Then, later, under the influence of gravitation the apex directs itself vertically (see Sachs, *Flora*, 1873). In making the observations it is necessary to sketch at intervals the form of the curvature of the shoot for the time being.

It is also seen that the curvature at the close of the geotropic nutation is very sharp. In earlier stages of the nutation we see particularly clearly that the curvature does not correspond with the arc of any circle, but on the contrary at a particular place is found a very sharp curvature (with very small radius), from which point, both backwards and forwards, it diminishes.

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[1] See especially Sachs in *Arbeiten des botan. Inst. in Würzburg*, Bd. 1, and H. de Vries in *Landwirthschl. Jahrbücher*, Bd. 9.

174. The Causes of Geotropic Curvatures.

The attraction of the earth is, of course, not to be regarded as the source of the force which performs the internal and external work in connection with geotropic curvatures. That force is furnished on the contrary by the plant itself, and gravity, which acts as a stimulus, only liberates it under certain conditions.

That a large amount of external work is done when negative geotropic curvatures take place is at once clear, if we bear in mind that the shoot-ends, often possessing no inconsiderable weight, are raised in the process. But even in the case of positive geotropic nutations the structures are not simply passively dragged down by their weight, but themselves actively participate in the movement which takes place. This is clearly shown by the fact that roots performing positive geotropic curvatures will force their way into mercury (see Fig. 145). Into a vessel of about 10 cm. diameter pure mercury is poured to a depth of 3 cm.

We now fix on one side, by means of shellac or otherwise, a piece of cork. To this is attached by means of a long pin a seedling of Vicia Faba or Phaseolus with a root a few centimetres in length, in such a way that the terminal part of its root rests horizontally on the mercury. After pouring some water on to the surface of the mercury we cover the apparatus with a bell-glass and let it stand. After some time (perhaps twenty-four hours), apart from secondary phenomena, it can be made out that the root-end has forced itself into the mercury. The geotropically curved root overcomes the resistance presented by the mercury, and grows vertically downwards into it (see Fig. 145).

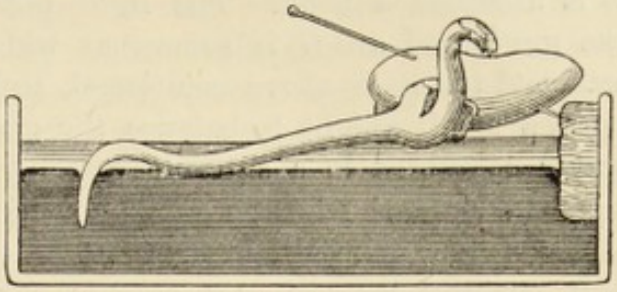

FIG. 145.—Seedling of Vicia Faba, whose root is forcing its way into mercury. (After Sachs.)

It can also be demonstrated by means of the apparatus depicted in Fig. 146 that roots execute their downward geotropic curvatures with great force. On the board *B* stands the metal pillar *S*. The metal bar *St* is movable on *S*; it is also

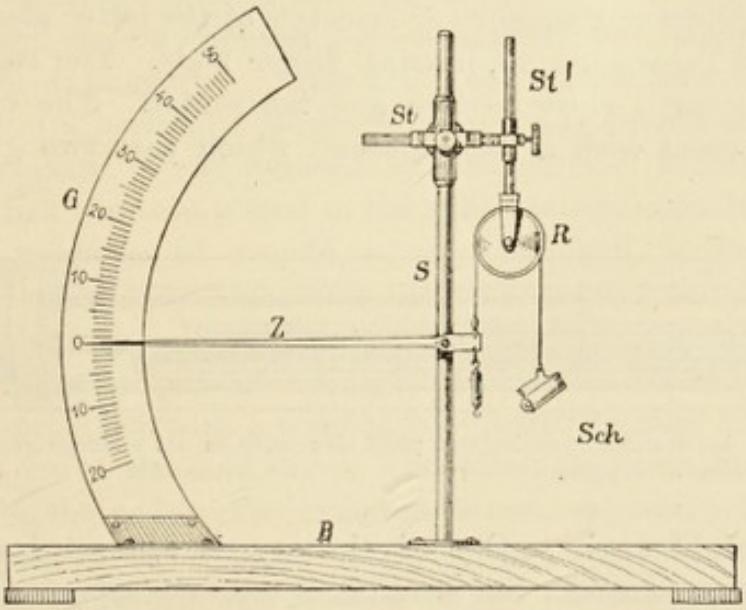

FIG. 146.—Apparatus for demonstrating that roots executing geotropic curvatures can do work.

capable of movement in a horizontal direction from right to left. To fix it in position there are screws. At one end *St* carries the rod *St*[1], movable upwards and downwards. This supports the

lightly running pulley *R*, over which passes a thread. At one end of this is suspended the small glass *Sch*; to the other end of it is attached a hook. The light pointer *Z*, which moves over the graduated arc *G*, is somewhat widened at the base. Here is attached the hook above mentioned, and also another which supports a small weight to balance the glass *Sch* when it dips into water contained in a suitable vessel placed below it. We now suspend from the hook a further weight, *e.g.* about 1 gr., if we experiment with seedlings of Vicia Faba. The seedlings are suitably attached by means of pins to a piece of cork fixed in the water vessel. The downward growing mainroot, whose tip is introduced into *Sch*, exerts on this a pressure, which forces it down in opposition to the weight attached to the other end of the thread, and the pointer *Z* consequently changes its position.[1]

We know—and we shall return to this point later—that the geotropic curvatures (apart from the geotropic movements of variation) are due to processes of growth. Most plants do not grow when deprived of free Oxygen, and therefore, in a medium free from Oxygen, the geotropic nutations also are arrested. This is easily determined by means of the apparatus illustrated in Fig. 147. Into the glass cylinder we bring a thin strip of wood *B*, soaked by boiling in water, and on this, by means of pins, we fasten seedlings of Pisum or Phaseolus or epicotyls of the latter plant, or, *e.g.*, scapes of Taraxacum, *St*, bearing flower buds. The regions capable of growth must be free and horizontal. The cylinder is tightly closed with a cork through which pass two glass tubes

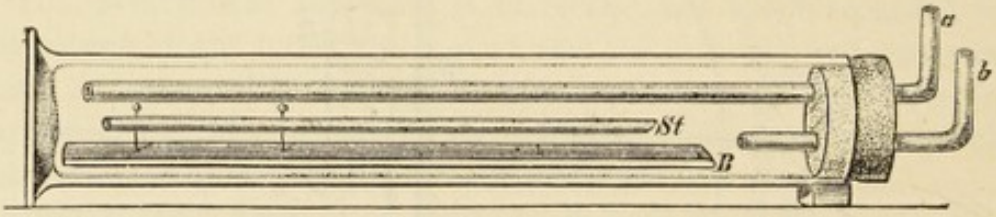

FIG. 147.—Apparatus for proving that plant structures do not undergo geotropic curvatures in absence of Oxygen.

bent at right angles. One of these, *a*, is connected up with a Hydrogen apparatus; the other, *b*, is the exit tube for the gases leaving the cylinder. For an hour we pass a stream of moist Hydrogen through the apparatus, but no geotropic curvature can be perceived when the cylinder, after standing for a time vertically (for about half an hour after starting the current of Hydrogen), is laid in a horizontal position. In a similar cylinder, seedlings or

shoots exposed to atmospheric air rapidly execute energetic geotropic nutations, especially if the temperature is high.[2]

Researches have led to the result that when gravitation acts as a stimulus it does not directly influence the growth of the organs, but first affects the turgidity of their cells. We experiment with the epicotyl of Phaseolus, with young shoot ends, 20 cm. in length, of Aristolochia, Taraxacum, Plantago, Papaver, etc. The objects are laid horizontally in a zinc box (see 173), so as to be in darkness and in a moist atmosphere. When, after a time (two to four hours) a distinct geotropic upward curvature is to be seen in them, we lay them on a card marked with a series of concentric circles. We seek the circle whose curvature coincides most nearly with that of the object, and observe its radius. We now lay the object in a 20 per cent. solution of common salt contained in a crystallising glass. After some hours we place the dish on the card, and, moving the limp plasmolysed structure with the forceps, once more find a circle most nearly corresponding with its curvature. The radius of this circle is greater than that of the one found before plasmolysis. We see that the geotropically curved structure retains a curvature after plasmolysis, and this is due to processes of growth brought about by the influence of gravity. That portion of the original curvature, on the other hand, which can be removed by plasmolysis, must be ascribed to conditions of turgidity. After the objects have been left in the zinc box, in a horizontal position, for perhaps twenty-four hours, their curvature is not reduced by plasmolysis; it remains unaltered when they are placed in the salt solution, since it has been fixed by processes of growth in the cells, and is therefore no longer reversible.

If we place in our zinc box in a horizontal position pieces of grass haulms (I experimented, *e.g.*, with Secale), 10 cm. in length, and each provided with a node at its centre, sticking their lower ends into one of the sand slopes, a considerable curvature quickly appears in the node. For example, in one case which I observed it amounted to 45°. After completely plasmolysing the piece of haulm the curvature was reduced to 25°. Unmistakably, therefore, the co-operation of conditions of turgidity and growth in the production of the geotropic curvature is here also to be recognised.[3]

From what I have stated in my *Lehrbuch der Pflanzenphysiologie* concerning the fundamental causes of geotropic nutations, it may be

maintained that gravity does not modify the osmotic pressure of the cells in the region of curvature, but modifies the power of resistance of the turgor-stretched cell layers (protoplasm and cell membrane). In negatively geotropic organs, the resistance of these layers in the cells of the under side, which is becoming convex, is lessened; that of the cells of the upper side, which is becoming concave, is increased. Consequently, the osmotic pressure remaining the same, the turgor-expansion of the cells of the lower side will be increased, that of the cells of the upper side diminished, and so we have at once the conditions for a difference in the growth of the opposite sides of the structure. Presumably, also, the quantity of water in the lower half of a structure executing a negative geotropic curvature will be greater than that of the upper half, and indeed Kraus[4] asserts that he has demonstrated such differences in the distribution of the water. Renewed researches, however, are required concerning the distribution of water in geotropically curved plant organs, since at any rate the results obtained by Kraus respecting the distribution of water in heliotropic stems have not been confirmed by Thate's observations.[5]

That in fact the osmotic pressure of the cells of the opposed sides of geotropically curved organs is the same, as above indicated, may be shown experimentally by the plasmolytic method.[6] Bean epicotyls grown in the dark are laid horizontally. If, after upward curvature has taken place, we remove thin sections of the cortex of both convex and concave sides, and examine microscopically, we shall observe the following. If the sections are laid in 2 per cent. solution of Potassium nitrate, no plasmolysis is as yet exhibited. Plasmolysis only appears when we work with about 2·5 per cent. solution of Potassium nitrate, and a solution of particular concentration acts in the same manner on the cortical cells, both of the convex lower and concave upper sides of the curved epicotyl.

If differences in osmotic pressure on the opposite sides of curved plant organs are not to be regarded as the cause of the nutations, the cause is probably to be sought in a special behaviour of the membranes. Wortmann[7] has endeavoured to support this view by the following instructive experiment. A pot plant of Phaseolus with an actively growing epicotyl is laid horizontally. Round the tip of the stem is fastened a silk thread, which passes over a lightly running pulley, and carries a weight sufficient to prevent

the epicotyl from executing any geotropic curvature. After thirty to forty-eight hours we cut thin transverse sections from the growing region of the epicotyl, and find on examination that the cortical cells of the upper side are very rich in plasma, have narrow lumina, and membranes strongly thickened in a collenchymatous manner, while, on the contrary, the cortical cells of the lower side are poor in plasma, have wide lumina, and thin walls.

In the experiments with the bean epicotyl, the geotropic curvature of which was prevented in the manner indicated, we naturally give the plasmic structures of the cells time to execute fully and completely the movements of irritation due to the action of gravity. These movements consist in a migration of the plasma from the lower to the upper side of the stem. The course of migration will obviously be along the plasmic connections between the cells. The significant accumulation of plasma in the cells of the upper side of the stem has here resulted in great thickening of the membranes and consequent feeble surface growth. On the under side of the stem is exhibited trifling growth in thickness, but vigorous surface growth of the cell membranes. Thus here the expansion of the cells under osmotic pressure may easily be considerable. According to Wortmann all the irritable movements of growing organs are brought about primarily by the migration of the plasma under the influence of the stimuli, this in turn influencing the growth of the cell membranes. The facts determined by this observer are undoubted, although as regards their interpretation there is no agreement as yet.[8] We cannot, however, pursue the matter further here.

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[1] Very beautiful and searching investigations respecting the pressure which may be exerted by growing plant organs, and the work which they can do, have recently been made by Pfeffer. See *Abhandlungen der Königl. sächs. Gesellsch. d. Wiss.*, 1893, Bd. 20. I am sorry that I could not give more time to this paper.

[2] See G. Kraus, *Abhandlungen der Naturforschenden Gesellschaft zu Halle*, Bd. 16, and Correns, *Flora*, 1892.

[3] See H. de Vries, *Landwirthschaftl. Jahrb.*, Bd. 9, p. 500.

[4] See G. Kraus, *Abhandlungen der Naturforschenden Gesellschaft zu Halle*, Bd. 15.

[5] See Thate in Pringsheim's *Jahrbücher*, Bd. 13.

[6] See Wortmann, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 5, p. 461.

[7] See Wortmann, *Botan. Zeitung*, 1887, p. 819, and 1888, p. 488.

[8] See especially Pfeffer, *Abhandlungen der Königl. sächs. Gesellsch. d. Wissensch.*, Bd. 18, p. 240.

175. The Function of the Root Tip in Connection with Geotropic Curvatures.

As is well known, it was asserted with great positiveness by Darwin that the root tip is of essential importance in connection with the production of geotropic curvatures in the root.[1] For this and other reasons he speaks of a "brain function" of the root tip, certainly an unfortunate expression, which may very readily lead to misconception. The question raised by Darwin, which, however, had already been investigated by Ciesielski and Sachs, has given rise to numerous researches, the results of which are partly for and partly against his view.[2] Seedlings of Pisum, Zea, Vicia Faba, or Phaseolus serve best for our experiments. After being soaked the seeds are germinated in moist sawdust until their roots, growing vertically downwards, have attained a length of 2–3 cm. Some of the roots (it is best to use a good number, say twenty) are now marked with ink lines at a distance of 15–20 mm. from the apex, and half of the seedlings are placed in moist sawdust with their roots directed horizontally. In the case of the remaining seedlings we remove the root tip to a length of 1·5–2 mm., by placing the root on a sheet of cork, and with a sharp razor cutting off transverse slices as nearly as possible at right angles to the root until the desired result is attained. The seedlings with the decapitated roots are now also laid horizontally in moist sawdust. After twenty-four to forty-eight hours we determine the amount of growth in all the seedlings, and observe whether they have performed geotropic curvatures. I found in experiments with Phaseolus multiflorus that the normal roots in the course of forty-eight hours had all made far greater growth than the decapitated ones. The root tips had been removed to a length of 2 mm. This result does not, however, harmonise with the statements of all, though certainly with those of some observers who have investigated the matter. My experiments with Phaseolus showed, moreover, that the roots of the intact plants executed normal geotropic curvatures, but that the curvatures made, it is true, by the decapitated roots were in different directions, sometimes upwards, sometimes sideways, or again downwards.

I am far from wishing to draw from my observations conclusions respecting the function of the root tip in connection with geotropic curvatures, since my experiments with Phaseolus, and

similar ones with Vicia seedlings have not been sufficiently numerous to justify this. In experiments with Vicia Faba, moreover, I found that during the first twenty-four hours the decapitated roots grew just as actively as the intact ones. The various questions here under consideration must not as yet, according to my own experience, be considered solved. The latest work of Pfeffer I have been obliged to leave out of consideration.

In going through the literature respecting the function of the root tip one is surprised at the number of contradictions in the statements as to its significance in longitudinal growth and geotropic curvatures. It appears to me that many of the experimenters in their investigations have not paid sufficient attention to certain considerations. (1) It is possible that the root tip has not the same importance as regards the growth and geotropism of the root in different species of plants. (2) It is necessary, in order to attain trustworthy results, always to work with a large number of objects. (3) It is not a matter of indifference whether we remove lengths of 1, 1·5, or 2 mm. from the end of the root, since frequently, when the decapitation is inconsiderable, the whole of the root tip, by which, according to Darwin, the stimulus is conveyed, will not be removed. (4) In prolonged investigations, attention must be paid to processes of regeneration taking place in the decapitated root.[3] (5) With reference to the nutation of roots (see 157), we have to lay stress on the position in which the intact and decapitated roots are disposed.

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[1] See Darwin, *Movements of Plants.*

[2] See Wiesner, *Bewegungsvermögen der Pflanzen*, 1881; Detlefsen, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 2; Kirchner, *Programm. zur* 64. *Jahresfeier der Akademie Hohenheim*, 1882; see further statements by Krabbe, Molisch, Wiesner, and Brunchorst, in the first and the second Jahrgang of the *Berichte der Deutschen botan. Gesellschaft.*

[3] In this connection see Prantl, *Arbeiten d. botan. Inst. in Würzburg*, Bd. I.

176. Experiments with the Clinostat.

The clinostat, which was introduced by Sachs,[1] is one of the most important instruments for making investigations in plant physiology. It enables us to prevent heliotropic and geotropic curvatures in plant structures.[2] An excellent form of clinostat was designed by Pfeffer, and this can be obtained at a price of

320 marks from Albrecht, Tübingen (see Appendix). The high price of the instrument[3] induced Wortmann to have clinostats constructed in a much cheaper form (200 marks) by Ungerer Bros., Strassburg.[4] This Wortmann's instrument I have frequently employed, and can recommend. It is certainly not so good as the large apparatus of Pfeffer, but may be employed in most cases. The following is a description of it:—

The whole clinostat, as represented in Fig. 148, consists of two parts, a driving mechanism and the attachments, which latter are essentially of the construction described by Pfeffer. The driving mechanism, *A*, is screwed on to a strong stand, *B*, and consists firstly of a clockwork, which is regulated not by an anchor [lever], but, in order to secure perfectly smooth rotation, by a fan [shown in Fig. 149]; and secondly, of three spindles, *a*, *b*, and *c*, standing vertically over one another, which, themselves freely movable, can by means of a push knob, *d*, be put in gear with a wheel of the clockwork, as shortly to be described, and thereby set in rotation. The arbor, *e*, receives the key for winding up the clockwork.

The attachments consist firstly of a solid shaft, *f*, which, by means of a joint, *g*, can be attached to any one of the spindles *a*, *b*, *c*. This joint enables the shaft *f* to be moved either in a horizontal or vertical direction, and so, without disarranging the whole apparatus, we can on the one hand rotate the research objects with the axis of rotation horizontal or inclined at any desired angle up to 45° with the horizontal, and on the other hand secure different positions with respect to the incident rays of light. On the shaft *f* is fixed by means of a set screw a movable ring, carrying a pin, *h*, on which slides the weight *i*. The pin and weight constitute a centering arrangement; they provide a means of counterbalancing any overweight, on one side or other of the axis, of the objects under rotation, which might seriously interfere with the regularity of the movement. The shaft *f* is supported at *a* on two friction rollers, which are capable of movement in a vertical direction—upwards and downwards—on the pillar, *k*, of a firm stand, and also capable of rotation round the horizontal axis, β, so as to accommodate the shaft *f* when it is inclined to the horizontal.

To the end of the shaft *f* is attached the flower-pot holder *l*. This consists of a three-limbed brass base, into the segments of which are rivetted at right angles three iron rods. Each of these

carries a movable brass triangle which, as is seen from the drawing, can be firmly fixed against the rim of the flower-pot, which is thus rendered immovable.

If now an object, say a pot plant, is to be rotated, we proceed

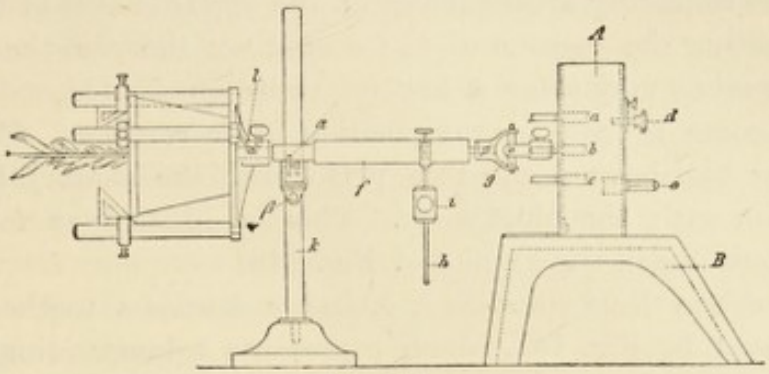

Fig. 148.

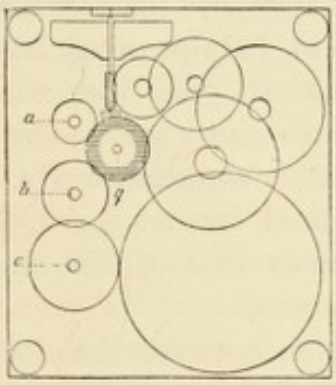

Fig. 149.

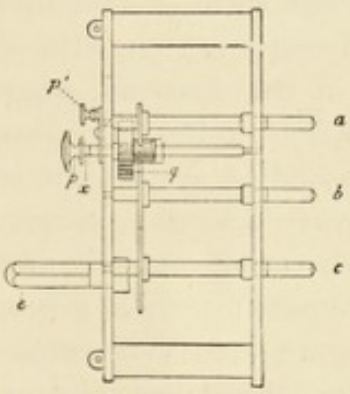

Fig. 150.

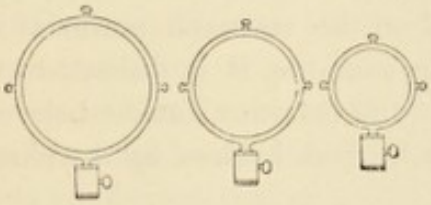

Fig. 151.

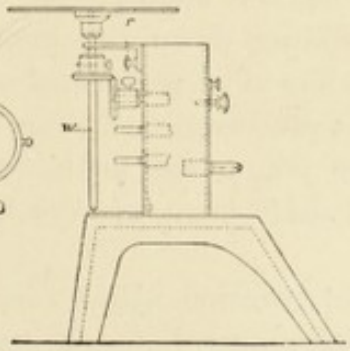

Fig. 152.

FIGS. 148-152.—Wortmann's clinostat with attachments.

as follows. The pot is first introduced into the support *l*, and fixed by means of the triangles, this operation being completed in less than a minute. The pot support is now fixed on to the shaft *f* by means of the set screw, and set in rotation by a twist of the hand (say against the pin *h*, from which the weight *i* must previously have been removed). If now the flower-pot has been

so fixed in the support that the weight is in excess on one side of the axis of rotation, then a particular side of the pot will always be found at the bottom when the apparatus comes to rest, the centre of gravity lying below the axis of rotation. By now fixing the centering arrangement on the opposite side of the shaft, and adjusting the position of the weight on the pin, the effect of the inequality may after a few trials be eliminated, so that the pot will come to rest in any position after rotation. When the centering has been accurately performed, the shaft *f* is put in connection with the clockwork. This is effected as follows by simply pushing in the knob *d* of Fig. 148.

Each of the three spindles *a*, *b*, and *c* carries a toothed wheel, as indicated by Fig. 149, which represents a longitudinal section through the clockwork. The toothed wheels of the spindles *b* and *c* engage with one another always; that of the spindle *a* does not directly engage with them. By pushing home a wheel, *q*, shaded in the drawing, whose teeth gear into the wheels of the two upper spindles, and also into one wheel of the clockwork, all three spindles are set in motion simultaneously. Fig. 150, which represents another longitudinal section through the clockwork in a direction at right angles to that of 149, shows clearly how the connecting wheel *q* is operated.

In the figure there is no connection between the clockwork and the spindles, the connecting wheel *q* not being in gear. By pressing on the knob *p*, the connecting wheel is pushed home, and by sliding down the knob *p'*, it is held in place. If any operation has to be performed on the research material while the rotation is proceeding,—if, for example, it is desired to water the soil in the pot and so forth,—the communication between clockwork and shaft must always be first broken by drawing out the pin *p*.

To effect rotation about a horizontal or inclined axis, but with the rotating object disposed in a direction at right angles to that indicated in Fig. 148, we fix the flower-pot by means of wire into one of the three rings represented in Fig. 151. For inclined axis of rotation the friction rollers are fixed on a second very strong stand.

If we are concerned with observations on objects requiring to be kept in a moist space, such as say fungi (sporophores of Phycomyces), roots, disconnected plant structures, etc., we may fix them on a brass axis 80 cm. long (not represented in the figures)

which is led through a glass case containing moist air, and whose extreme end, outside the case, is then supported by the stand with the friction rollers.

If it is required to rotate the objects about a vertical axis, the clinostat must be arranged in a different manner, since the driving apparatus is not movable.

Fig. 152 shows the clinostat arranged with the axis of rotation vertical. A bevel wheel is put on one of the spindles *a*, *b*, *c*, the teeth of which gear into those of a second bevel wheel. The latter can be fixed in any desired position on a vertical shaft, *w*, which rests on a steel bed, carries at the top a flat brass disc, and by means of a set screw, *r*, can very quickly be clamped to the driving apparatus.

The rates of rotation of the three spindles are different, and so arranged that without loading, and with the axis of rotation horizontal, one revolution of *a* is made in ten minutes, of *b* in fifteen minutes, and of *c* in twenty minutes. But if it is required, greater rapidity may be secured by closing the fan.

The clockwork requires winding up every twenty to twenty-one hours.

The advantages of the clinostat are as follows:—

(1) In rotation all possibility of shocks is eliminated, so that a perfectly even movement of the objects is rendered possible.

(2) It is very convenient for transport, and can hence be set up on any work-table.

(3) Since the clockwork is almost noiseless in its movement, the apparatus is suitable even for lecture demonstrations.

(4) The manipulation is exceedingly simple. The material for observation can in the course of one to two minutes be mounted, attached to the apparatus, and set in movement. The removal of the objects is effected just as simply.

(5) The strength is ample for ordinary experiments. Tests showed that with the axis of rotation horizontal (as represented in Fig. 148), and with a load of 2 kg., the movement was still perfectly smooth and regular, though certainly it was retarded by one minute per revolution. With the axis vertical, however (as represented in Fig. 152), a load of 5 kg. could be employed without retarding the rotation.

(6) The price of the apparatus, including accessories, is 200 marks.

In order to obtain a general idea of the efficiency of our appar-

atus, and to study the behaviour of many plant organs under different forms of rotation, we make the following experiments. We put the clinostat in front of the window, and on the disc (see Fig. 152) rotating horizontally about a vertical axis, place a flower-pot containing a few very sensitive heliotropic seedlings (Sinapis, Lepidium) which have just emerged from the soil, and which have hitherto been kept in the dark. As long as the rotation continues, these seedlings undergo no heliotropic nutations, in spite of the one-sided illumination, while similar objects not rotated all curve over towards the window.

In order to eliminate, in experiments with seedlings, etc., the one-sided action of gravity, the axis of rotation must be accurately horizontal (see Fig. 148).

Rotation in the vertical plane taking place at the rate of one revolution per ten to fifteen minutes is slow enough to prevent any effect of centrifugal force. Above all, it is, however, exceedingly important, in order to secure uniform rotation, that the loading of the axis shall be uniform all round. This can be effected by means of the pin *h* and the weight *i* (see Fig. 148). The seedlings themselves, *e.g.* maize, pea, or bean seedlings, grown in sawdust, are placed in a drum constructed out of thin sheet zinc. This is put in the pot-holder in place of a flower-pot, and suitably fixed. The base of the drum is formed by a piece of soft wood, so that the long pins which carry the seedlings may be fixed without difficulty. The sides of the drum are covered inside with several layers of moistened blotting-paper, as also the non-growing parts of the seedlings. On the base of the drum we spread moist cotton wool, which is held in place by means of small pins. It is also very convenient to experiment as follows. We connect up one end of a shaft about 80 cm. in length with the clockwork of the clinostat, while the other end rests on friction rollers. We now slip a tightly-fitting cork over the shaft, so that it can be rotated like a wheel in the vertical plane. The seedlings are then fixed on the periphery of the cork with two pins each, so that the weight is distributed as evenly as possible. Under the cork stands a reservoir of water, so that the plants at each revolution of say twenty minutes dip for a while (one to two minutes) into water. By covering with a glass box, provided with corresponding slits for the shaft, the air in the neighbourhood of the seedlings is kept moist. The whole apparatus is put in a dark chamber.

The objects (*e.g.*, four seedlings of Phaseolus, Pisum, or Vicia Faba) are fixed on the cork, when the main root has just emerged. We fix them with their main roots making different angles with the axis, and find after some days (it is best to experiment at a comparatively high temperature, say 20° C.) that all the roots have lengthened in the original direction of growth, assuming uniform movement of the apparatus. Occasional curvatures may appear in the roots; they are not, however, geotropic nutations, all similarly directed, but spontaneous nutations.

As regards the lateral roots, these, after a time, grow out from the main roots of the seedlings which have been rotating from the commencement of germination. The angle which they make with the main root, the so-called "proper angle" of the lateral roots of the first order, is determined in our experiment by internal causes alone. This proper angle is in different objects, and in the individual lateral roots of a particular main root, by no means always the same. In general it happens, as can easily be made out by measuring the angles, that the lateral roots at the base of the root are directed almost or exactly at right angles to the main root, while the later ones have an acute "proper angle"; they are inclined towards the apex of the main root. Frequently also the lateral rootlets bend in the form of an arc. Seedlings of Phaseolus are particularly suitable for investigations of this kind. In this plant lateral roots are also usually given off by the short hypocotyl, which, as must be specially mentioned, exhibit an obtuse "proper angle."

In order to subject plants to one-sided illumination, but eliminate the one-sided influence of gravity, the axis of the clinostat must be directed parallel to the incident rays of light. The plane of rotation of the objects must make a right angle with the rays of light. The pots, in which the plants grow, are fixed in one of the rings depicted in Fig. 151, in the manner already described on p. 456. For more detailed information see 178.

A cube of bread, with sides 4–5 cm. in length, is moderately (not too much) moistened with water. It is best to sterilise the cube while moist by exposing it for some hours in a crystallising glass covered with a sheet of glass to a temperature of rather more than 100° C. in a drying chamber. The cube is now pushed over the brass shaft (80 cm. in length) of the clinostat, one end of which communicates with the clockwork, while the other rests on the friction rollers. The shaft is perfectly horizontal and

parallel with the window panes. The cube is held securely at the middle of the shaft by means of tightly fitting corks slipped on to the shaft to the right and left of it. Below the shaft of the clinostat is a zinc dish, 50 cm. long in the side, containing water. A glass case traversed by the shaft, and standing in the water, serves to keep the air about the bread moist. The case has a zinc framework; this, on the two sides where the shaft passes through it, possesses slits, which when the apparatus is completely put together can be sufficiently closed. The front and back, and also the roof of the case, is formed by panes of glass.

To make an experiment, some sporangia of Mucor or Phycomyces nitens are distributed in sterilised water. By means of a flat needle, sterilised by heating, we sow all six faces of the cube of bread with spores, and after covering with the glass box, at once set the clinostat in rotation. After a few days the sporangiophores rise from the substratum, and quickly attain a considerable length. The sporangiophores on the flanks of the cube, which we shall not further consider, are certainly somewhat curved, since from time to time, at each revolution, they come into the shade of the shaft, whereby heliotropic curvatures are occasioned. On the other faces of the cube, however, the sporangiophores are straight; they are directed at right angles to the substratum.* This relation of the organs to the substratum, which is certainly not, as supposed by some physiologists, caused by the mass of the bread, comes about in rather a complicated manner.[5]

It cannot be due to geotropism, since, of course, the one-sided influence of gravitation is eliminated by the rotation of the object on the clinostat.† But negative hydrotropism, which will be more closely considered below, is of importance, and the capacity of the sporophores to re-act by irritation curvatures to the stimulus of contact,[6] and of one-sided illumination, also demands attention.

We can easily convince ourselves of the heliotropic irritability

* The sporophores on the edges of the cube are disposed in a direction which bisects the angle made by the corresponding faces.

† That does not say, of course, that the sporophores of Mucor are not geotropically irritable. They are highly irritable. If, *e.g.*, a cube of bread, sown with spores of Mucor, is stuck on a long pin and suspended by means of it in a large glass cylinder, whose base is covered with water, then in a few days, the cylinder being kept in the dark, erect sporangiophores spring from the upper face of the cube. The sporangiophores which develop on the flanks of the cube arch upwards, exhibiting negative geotropic irritability. From the base of the cube grow branched mycelial threads, which exhibit positive geotropism.

of the structures by illuminating from one side in a heliotropic chamber (see 178) straight sporangiophores grown on cubes of bread in the dark. And if now, in clinostat experiments with access of light, the sporangiophores grow out obliquely to the surface of the cube of bread, the side which they turn towards the face of the cube, whether that face is directed towards the window or away from it, is always more feebly illuminated than the opposite one. Heliotropic effects must therefore follow, and these, like the hydrotropic processes which are certainly of primary importance, would bring the sporangiophores into a direction at right angles to the substratum.

We now fix on the shaft of the clinostat soaked cubes of turf (length of side about 5 cm.), and sow them with seeds of Lepidium sativum or Sinapis nigra, leaving the two flanks unsown. The seeds cling to the wet turf, and do not require specially fixing. The clinostat, with the glass case *in situ*, is placed with its shaft parallel to the window panes, exposed to bright diffused light, and set in motion. After several days we observe that the developing roots, owing to their positive hydrotropism, are clinging tightly to the moist substratum, even penetrating it in part. The hypocotyls at first vigorously nutate; soon, however, they take up a position at right angles to the surface of the turf. This, as was shown by Dietz, is not due at all, or only in a subordinate way, to hydrotropism, or contact stimulus. Here we really have to do with heliotropic effects, brought about in fact in the same manner as was described in the case of the sporangiophores of Mucor. If, viz., we allow cubes of turf, sown with Lepidium or Sinapis, to rotate slowly about an horizontal axis in the dark, then the hypocotyls do not stand at right angles to the substratum, but assume the most various directions. Seedlings of Phleum pratense are also very suitable for experiments with the clinostat, but they do not grow so rapidly as, *e.g.*, seedlings of Lepidium. If we sow cubes of turf standing in water with seeds of Phleum, and cover with a cardboard box, the plumule of the seedlings on the horizontal upper surface grows vertically upwards. The plumule in the seedlings lying on the vertical sides of the cube arches upwards, owing to its great geotropic irritability.

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[1] See Sachs, *Arbeiten des botan. Inst. in Würzburg*, Bd. I., p. 597, and Bd. II. p. 215.

[2] Sachs and Noll described the special behaviour of dorsiventral organs when rotated on the clinostat (*Flora*, 1893).

[3] Albrecht also supplies a clinostat for 220 marks.

[4] See Wortmann, *Berichte d. Deutschen botan. Gesellschaft*, Bd. 4.

[5] See Sachs, *Arbeiten des botan. Inst. in Würzburg*, Bd. 2, p. 217; and Dietz, *Unters. a. d. botan. Inst. zu Tübingen*, Bd. 2, p. 478.

[6] See Wortmann, *Botan. Zeitung*, 1887, No. 49.

177. Experiments with the Centrifugal Apparatus.

I will first describe a form of centrifugal apparatus designed by myself, and depicted in Fig. 153, which can be very easily and cheaply constructed, and which is very suitable for demonstration purposes. The most important part of the apparatus is the brass shaft *A*, about 50 cm. in length, which carries the zinc disc, *Z*, 40 mm. in diameter, on which are soldered the six metal vanes, 3 cm. wide and 7·5 cm. long. At the opposite end

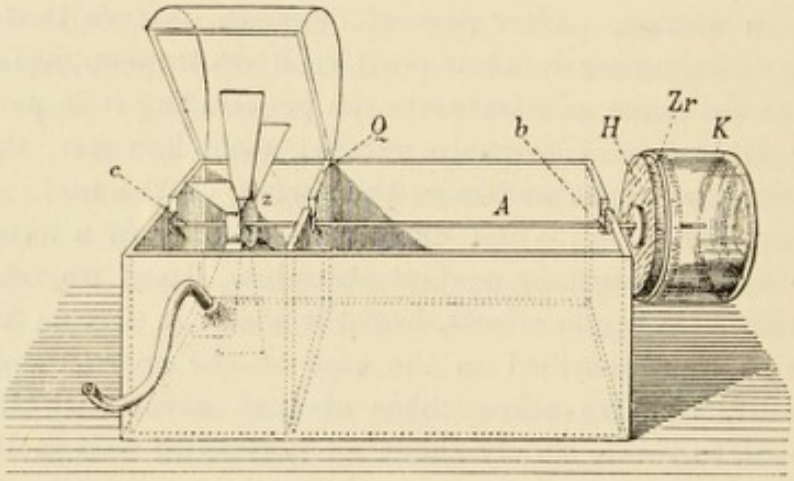

FIG. 153.—Centrifugal apparatus.

of the axis is fixed a disc of wood, *H*, 140 mm. in diameter, to which is attached a sheet of cork. The zinc ring, *Zr*, is fixed to the margin of the disc of wood, but projects beyond it, and may be employed to give support to a zinc cylinder about 11 cm. in length, closed at one end, and serving as a cover, so that, *e.g.*, seedlings, fixed to the cork with pins, are excluded from the light when the cover is put on. In the drawing the wooden disc, *H*, carries a bell-glass, *K*. The shaft *A* rests at *b* in a bearing. At *c* is a second point of support for the shaft, the end of which here forms a point. As our drawing shows, the arrangement described is supported by a box. This is about 15 cm. high, 27

cm. broad, and 41 cm. long. The walls of the smaller compartment in which the vanes are to move are lined with sheet zinc. In the front is an opening which is closed by means of a perforated cork. Through this passes a glass tube, the end of which towards the vanes is somewhat drawn out, while over the other end can be slipped a tube connecting with the water supply. By directing a stream of water on the vanes 200–300 revolutions per minute can be obtained if the force of water is fairly strong. To avoid scattering of the water the smaller compartment is provided with an arched wooden cover. The water dropping from the vanes is carried off by a hole in the bottom of the box direct into the sink on which the apparatus is placed.

Suitable objects for examination are, *e.g.*, pea seedlings grown in moist sawdust, and provided with roots about 2 cm. long. The cork which rests on the wooden disc *H* is soaked with water, and on it is placed wet cotton wool. This is fixed by means of pins. We attach the seedlings to the cork, likewise with pins, near its periphery, say with their main roots directed parallel with the axis of rotation. The zinc cover is now coated on the inside with moist blotting-paper and put on. In my investigations at about 20° C., the result was already very obvious after three hours' rotation. The growing root tips of the seedlings fixed to the disc of the apparatus in various directions had all curved outwards, so that they now formed a right angle with the axis of rotation (see Fig. 154). In experiments lasting for some time, which, *e.g.*, are intended for studying the behaviour towards centrifugal force of the secondary roots of seedlings, it is necessary to interrupt the experiments, say every three to four hours, in order to keep the cotton wool on the cork and also the seedlings sufficiently moist by renewed watering.

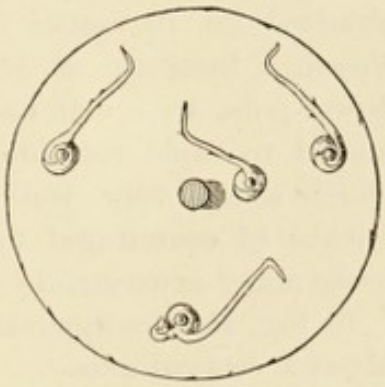

Fig. 154.—Seedlings of Pisum, whose root tips, owing to rapid rotation of the disc of a centrifugal apparatus, have curved about a horizontal axis.

The apparatus described is not strong enough to support flower-pots containing soil and seedlings. But in order to demonstrate the influence of centrifugal force on growing stems I proceed as follows:—

Two short test-tubes, about 55 mm. long and 25 mm. wide, are filled with moist sawdust. In this we lay a few seeds of Lepidium sativum. In the dark they rapidly begin to germinate, and when the hypocotyls, with their ends nodding, have emerged from the sawdust, the experiment can begin. To fasten the culture cylinders diametrically opposite one another, at the periphery of the cork, we slip them into pieces of cork perforated in the middle, and attach these by means of several pins to the large cork plate of the apparatus. After some hours the result of the experiment is clearly to be made out. The hypocotyls, directed at the beginning of the experiment parallel with the horizontal axis of rotation, have experienced a fresh curvature somewhat below the place at which they are curved owing to spontaneous nutation. The parts of the hypocotyl lying above this new zone of curvature have directed themselves towards the centre of the disc, not like the growing root tips towards the periphery of the disc. Seedlings of Triticum are also suitable objects for investigation.

I have now designed a large centrifugal apparatus (to be obtained for 70 marks from G. Tegetmeier, mechanician at the Physical Institute at Jena), by means of which, *e.g.*, even large flower-pots, with soil and plants growing in them, can be submitted to rapid rotation (*e.g.* 300 revolutions per minute), and which serves very well for thoroughly investigating the influence of centrifugal force on plants. I have indeed already made many experiments with this arrangement.

In Fig. 155 the apparatus is represented as arranged for rotation about a vertical axis.

On the stout board *B* is screwed a metal bracket, *M*. Alongside this is the support *Ah* for the shaft, which can be fixed rigidly to *M* by means of the set screw *Sch* and the pin *St* when the axis of rotation is required to be vertical. The shaft has a length of 20 cm. At *a* it rests on a pivot not visible in the drawing; at *b* is a bearing for the shaft. Over the grooved disc, *c*, which is attached to the shaft, can be passed an endless cord. The shaft support is perforated at *d*. In the perforation is inserted the lower end of the metal tube, *Mr*, which has a very fine opening. Into this tube we pour a little oil to ensure adequate lubrication of the bearing at high rates of revolution. Oil is also applied at intervals to the end *a* of the shaft. If the apparatus has not been in use for some time, it must be freed at *a* and *b*

from possibly dried up oil (the apparatus must be kept in a place as free from dust as possible). Petroleum is very good for the purpose.

To the shaft is now fixed the metal disc *Msch*, which is about 17 cm. in diameter. This disc, seen from above in Fig. 156, is provided in the neighbourhood of its periphery with three slots, in which the three flower-pot supports can move. Each of these

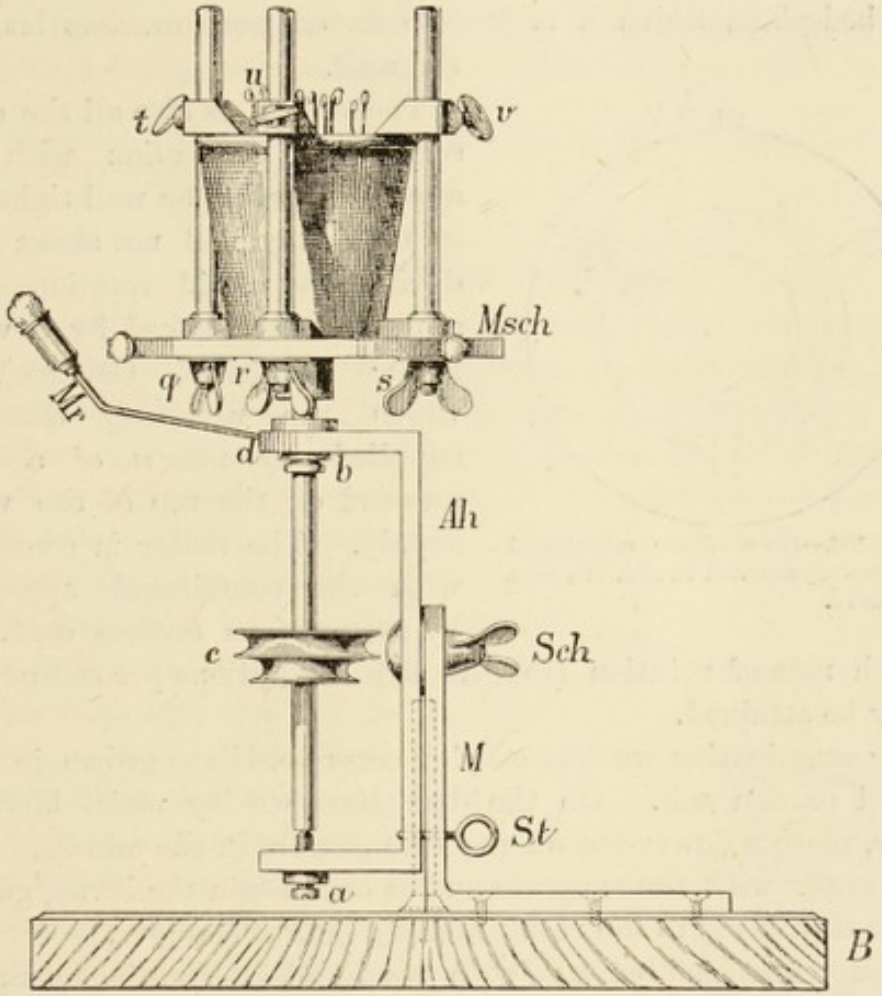

FIG. 155.—Centrifugal apparatus.

supports consists of a vertical rod, which at the base, as indicated in Fig. 156, carries a metal plate; a screw situated below the disc (the three screws are lettered *q*, *r*, *s*), and serving as a clamp; and a sliding piece (the three sliders are lettered *t*, *u*, and *v*), to fix the flower-pot after it has been placed on the disc. If the rotation is required to be in the vertical, instead of in the horizontal plane, so as to eliminate the influence of gravity, the shaft must be fixed horizontally. This is managed by loosening the set screw *Sch*, removing the pin *St*, turning the shaft

support through an angle of 90°, so that its end rests on the metal rod indicated in Fig. 155 by dotted lines, and standing at right angles to the board *B*, and then again tightening the set screw *Sch*. If the plants in the flower-pots are to be removed, during rotation, from the influence of light, we employ sufficiently deep sheet zinc cylinders, pierced near the rim with holes so that they may be fastened to the disc of the apparatus by the screws lettered *h*, *i*, *k*, in Fig. 156. In use the apparatus is screwed to a bed-plate, which it is best to fix on iron brackets let into the wall.

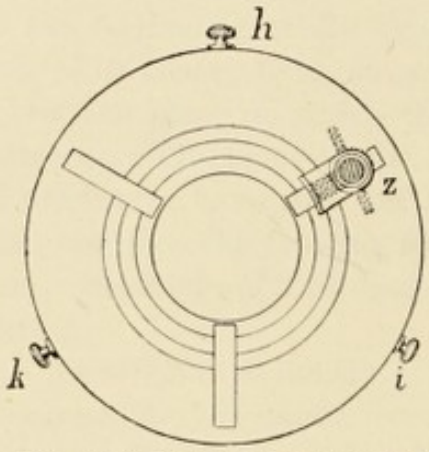

FIG. 156.—Disc of the centrifugal apparatus represented in Fig. 155, seen from above.

The screws, as also all the other screws in connection with the apparatus, must be well tightened so that they will not shake loose during the rapid rotation. The apparatus is worked by a water motor (Herbertz, Cologne), to which the necessary water is supplied by means of a tube screwed on the tap of the water supply. The motor is connected with the centrifugal apparatus by means of an endless cord, and a high rate of rotation (four to five revolutions per second) can easily be attained.

For examination we first employ cress seedlings grown in well-soaked garden soil. On the disc *Msch* we lay moist blotting-paper, place a flower-pot on the disc exactly in the middle, fasten it carefully with the supports and at once begin the investigation, which soon leads to the result already indicated above.

I also experimented, *e.g.*, with young wheat seedlings grown in pots. At a high summer temperature the plumule had already curved towards the centre of the disc after two to three hours' rotation. In experiments on the influence of centrifugal force on the growth of roots, it is best to proceed as follows. The pot supports are removed from the disc, and we place on it a zinc cylinder about 15 cm. long, pierced near the lower end with three holes, so that it can be fixed by means of the screws *h*, *i*, and *k*. Just above these holes is the bottom of the cylinder, which therefore rests directly on the disc *Msch*. The upper open end of the cylinder is provided with a cover. The inside of the

cylinder is covered with moist blotting-paper. On the bottom of the cylinder we fasten a sheet of cork soaked with water, and cover it with wet cotton wool. The seedlings are pinned to the cork as far as possible from the centre. Care must be taken that they are kept sufficiently moist. Seedlings of Pisum with roots about 2 cm. long are very suitable for investigation. To study the behaviour of secondary roots we employ seeds of Vicia Faba. Experiments made with the axis of rotation horizontal are continued for some time, and we find that the secondary roots under the influence of the centrifugal force all curve outwards.[1]

[1] See Knight, *Philosophical Trans.*, 1806, T. 1, p. 99; and Sachs, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 607.

178. Heliotropic Nutations.

Many plant structures, especially young stems, when exposed to one-sided illumination, bend towards the rays of light; they are positively heliotropic. Some structures, *e.g.* the roots of many Cruciferæ, are negatively heliotropic; under one-sided illumination, they curve away from the light. A small glass vessel, over the mouth of which is firmly tied a piece of small-meshed netting, is completely filled with spring water. Some seedlings of Sinapis alba, germinating in sawdust, are fixed in the meshes of the net by means of cotton wool. The vessel is then covered for a day with a bell-glass, and this with a cardboard box. As germination proceeds in the dark the roots grow straight down into the water, while the hypocotyls grow vertically upwards. In order now to submit our plants to the influence of one-sided illumination, we place the vessel with the seedlings under a beaker, covered, with the exception of a small slit, with dull black paper, or under a cardboard box provided with a slit, and covered on the inside with dull black paper. After a few hours it can already be clearly seen that the hypo-

Fig. 157.—Seedling of Sinapis alba. The hypocotyl exhibits a positive heliotropic curvature, the root a negative one.

cotyls of the seedlings have turned towards the rays of light passing through the slit, while the roots on the contrary have turned away from the light. In Fig. 157 is depicted a seedling whose hypocotyl has performed a positive heliotropic nutation, while its root has experienced negative heliotropic nutation.

Only comparatively few plant structures are negatively heliotropic. Positive heliotropism on the other hand is very common. Good research material is found in seedlings of Phaseolus multiflorus, Vicia sativa, and Lepidium sativum, grown in the dark. We cultivate the plants in small flower-pots, filled with loose garden earth, and when the epicotyls or hypocotyls as the case may be are in vigorous growth, expose them to unilateral illumination in the manner above described. The heliotropic nutations are soon exhibited, and often proceed till the ends of the shoots

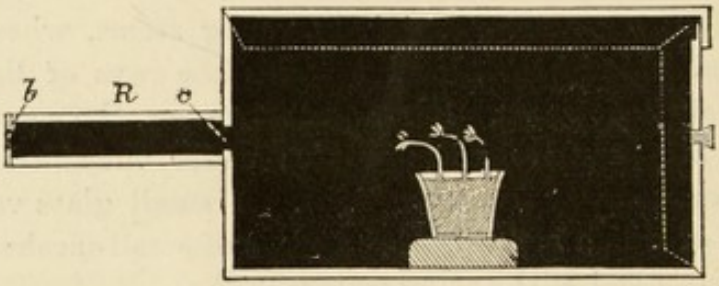

Fig. 158.—Heliotropic chamber.

are parallel with the incident rays of light. The heliotropic sensitiveness of different structures is, however, by no means the same. For example, the epicotyls of Vicia sativa, and plumules, say 2 cm. in length, of Triticum vulgare, are very sensitive, while the epicotyls of Phaseolus do not react so vigorously to the stimulus of light. If we cut young shoots of Sambucus nigra, and, after stripping off their leaves, place them with their lower ends in water, and then illuminate them from one side, we find that they only slowly bend towards the light. Their heliotropic irritability appears at all events to be insignificant, but further information can only be obtained by searching investigation with the clinostat, with elimination of geotropic nutations (see also below).

It is also very convenient, especially in demonstrations, to prove the occurrence of heliotropic nutations by means of a so-called heliotropic chamber. This consists of a box made of stout cardboard, about 16 cm. high, 20 cm. long, and 12 cm. broad (see Fig. 158). The box is covered inside with dull black paper, and

the cover and back can be folded back. The front of the box is provided with an opening $1\frac{1}{2}$ cm. wide, and carries the tube *R*. The tube is closed at the front by a plate provided with a slit. This slit is in a straight line with the slit *c* of the box. We raise seedlings of Sinapis or Lepidium in small flower-pots. When the plants have just emerged from the soil, we put one of the flower-pots into the heliotropic chamber, direct the slit *b* at the window towards white clouds, or towards a white wall illuminated by the sun, and even after a few hours we find that the plants have curved heliotropically. (See Sachs, *Lectures.*)

If seedlings of Lepidium sativum or other objects are very feebly illuminated, they curve only slowly towards the light. In investigations which I made with seedlings of Lepidium hitherto grown in the dark, the stems exhibited heliotropic nutations more rapidly when placed in diffuse light immediately in front of a window looking to the south, than when they were placed several metres from the window and illuminated unilaterally. On the other hand it appears that high intensity of light retards heliotropic nutations. (See also below.)

If seedlings grown in flower-pots (I used Lepidium) are placed, as described in 8, in a box in which they are exposed to mixed yellow light transmitted through a solution of Potassium bichromate, they do not curve towards the incident rays of light at all or only very slowly. In the experiment of which the result is illustrated in Fig. 159, the seedlings made no heliotropic curvatures at all. Wiesner observed, however, even in mixed yellow light, slowly appearing heliotropic nutations, a result justified by the conditions under which he experimented. Under the influence of the mixed blue light transmitted by an ammoniacal solution of Copper oxide vigorous heliotropic nutations take place.

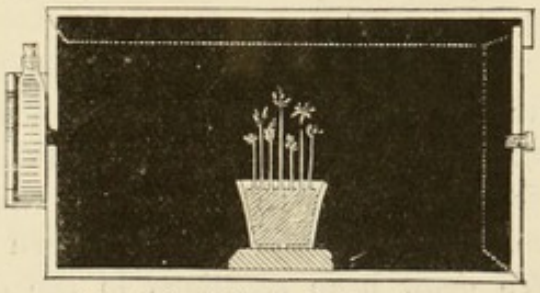

FIG. 159.—Heliotropic chamber.

In searching investigations as to the influence with respect to heliotropic nutations of rays of light differing in refrangibility, the use of the objective spectrum is strongly to be recommended. A heliostat set up before the window (on a firm support, so as to be free from vibrations) throws a beam of parallel rays through a

narrow slit into a dark room. After the beam has traversed a biconvex lens placed at a distance from the slit not quite equal to twice its focal length, it falls on the flint glass prism, which has an angle of 60°. Since glass vigorously absorbs the highly refrangible rays, it is very advisable to provide the heliostat with a silver mirror, and to use a quartz lens and a quartz prism.

We may employ for investigation, *e.g.*, vetch seedlings grown in the dark in very small earthenware vessels. They are placed within the spectrum in such a way that the flanks of the stems are directed towards the incident rays of light. Seedlings of Sinapis and Triticum also serve well for the investigations. In the course of a few hours the result of the experiment stands out clearly. Neglecting the so-called lateral flexion, it is seen especially that the rays at the limit between violet and ultra-violet display the greatest heliotropic energy. Towards very sensitive objects the heliotropic energy of the rays falls off from violet to green; yellow is inactive; a second smaller maximum lies in the ultra-red.

If we cultivate Mucor Mucedo on bread, in darkness, as described in 181, and then illuminate the culture unilaterally, the unicellular sporophores curve towards the light. They are thus positively heliotropic, which, as I specially set forth in my *Lehrbuch der Pflanzenphysiologie*, p. 308, is of significance in connection with the theory of heliotropism.

When heliotropic nutations are exhibited, the side of the structure which becomes convex always grows more strongly, in consequence of the light stimulus, than the side which becomes concave. Moreover, growing structures alone are capable of heliotropic nutations, as is shown by a very simple experiment. We grow bean seedlings in the dark. When the epicotyl has reached a length of some centimetres, we make fine ink-lines on the stem at intervals of about 5 mm., and illuminate unilaterally. The heliotropic curvature only appears in the upper, still growing region of the epicotyl.

If we illuminate etiolated vetch seedlings for about twelve hours unilaterally, and then cut off the epicotyls and place them in a 15 per cent. solution of common salt, the heliotropic nutation is not reversed again by the plasmolysis, since it has already been completely fixed by growth. If, however, we only continue the one-sided illumination long enough to make the epicotyl bend slightly, then the curvature is somewhat diminished by plasmolysing, since

at least that part of it can still be eliminated which is due to a difference in the expansion under osmotic pressure of the cells of the convex and concave sides of the structure, and which has not yet been fixed by growth.[1]

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[1] Numerous references to the literature respecting heliotropism will be found on pp. 303 and 304 of my *Lehrbuch der Pflanzenphysiologie*, Breslau, 1883. Many valuable details are given especially by Sachs and Wiesner, *Denkschr. d. Akad. d. Wiss. zu Wien*, Bd. 39 and 43.

179. Heliotropism (*continued*).

To study more exactly many of the phenomena associated with heliotropic nutations, we make the following experiments. We arrange our clinostat in a dark room, in such a way that its shaft is parallel to the rays of light entering by an opening in the window shutters. A flower-pot, in which a Phaseolus plant raised in the dark is growing, is fixed on the clinostat in the manner described on p. 456. The light rays must travel in a direction at right angles to the plane of nutation of the epicotyl, and this latter must still be in active growth, and is marked off into 5 mm. zones by means of ink-marks placed on the flanks towards and away from the light. If now we set the clinostat in movement, pure heliotropic nutations are exhibited. Geotropic nutations are eliminated, and moreover the spontaneous nutation of the epicotyl does not interfere. The heliotropic nutation does not of course appear at once, but after a little time. When it has become very pronounced we stop the experiment, ascertain by means of the cyclometer (a card marked with concentric circles of radii 1, 1·5, 2, etc. cm.) the radius of the curvature and determine the increments of growth. We find that the convex side of the epicotyl has grown much more than the concave side of it. Only the still growing parts of the epicotyl are capable of executing heliotropic curvatures. If the experiment is continued long enough, all the zones of the epicotyl which were in a state of elongation will take part in the nutation in succession.[1]

It was emphasized in 169 that the swarmers of Algæ are repelled by intense light, while they are attracted towards more feeble light. Hence it may be supposed that even the organs of higher plants are attuned to light of a certain definite intensity. Very thorough research, it is true, is still

necessary to set this conjecture on a firm basis; but the results of certain experiments of Oltmanns[2] are certainly in favour of it. If young seedlings of Lepidium grown in a flower-pot are placed in a box blackened on the inside, and provided with a slit about 3 cm. wide, and, after being pushed close up to the slit, are exposed to direct sunlight (we take care to rotate the box so as to have it always in approximately the same position with respect to the sun), then the hypocotyls remain straight, whereas in less intense light they exhibit positive heliotropism. We may further arrange an experiment in which the sun's rays are directed into a dark chamber, by means of a heliostat, through a thick layer of concentrated alum solution, and then passed through a large biconvex lens, behind which is a flower-pot with Lepidium seedlings. The seedlings are planted in a row, and this is presented to the rays diverging from the focus of the lens at an angle of about 45°. In this way one seedling stands nearly at the focus of the lens, the rest more and more remote from it without shading each other. With sufficiently strong illumination we find that the stems of the seedlings which stand nearest to the focus of the lens undergo negatively heliotropic curvature. The third or fourth seedling of the row remains quite straight. The fifth and sixth and seventh experience positive heliotropic nutations.

If we lay across the middle of a vessel containing algal swarmers a small strip of wood, so that it is directed at right angles to the window, and consequently nearly parallel to the incident rays of light, the swarmers do not collect at the front of the dish, but on either side of the strip of wood in its penumbra. Famintzin and especially Oltmanns (*Flora*, 1892, p. 203) conclude from this that the phototactic movement of the swarmers is determined not by the direction of the light rays, but by the intensity of the light or the rate at which it falls off. Oltmanns assumes that this is also the case in heliotropic nutations, and some investigations instituted by myself perhaps favour this view. The important question here touched upon requires, however, further very searching investigation, and in the following experiments, as in those above alluded to, it appears to me that very special attention must be paid to the effect of reflected light, in order to avoid errors in the conclusions. For my experiments I employed a box blackened on the inside, and having in one wall a slit of slight width but considerable length. Within the box, behind this slit, was placed a wedge-shaped glass bottle containing

water impregnated with Indian ink. The wedge-shaped cavity of the bottle, about 18 cm. long, was only about 3 mm. wide at one end, but more than 25 mm. wide at the other. If pots of seedlings were brought into the box, and this was disposed in the open air in such a way that the wedge lay horizontally, and the rays of light entered the apparatus from above, then all the seedlings curved towards that part of the box in which the intensity of the light was greatest, and consequently at right angles to the entering rays and towards that end of the box towards which was directed the narrow angle of the wedge. When the box was placed in the room with the wedge parallel with the window panes, and therefore approximately at right angles to the incident rays of light, while the pot was placed behind the wedge about centrally, then the hypocotyls did not curve exactly at right angles to the incident rays of light; neither did they turn directly towards the light, but curved obliquely towards the place where the intensity of the light was greatest. I employed Lepidium seedlings.

The following experiments on heliotropic nutations are also of great interest.[3]

We lay grains of Avena sativa in dishes containing a little water. When germination has just begun, we select seedlings very similar in appearance, plant them in good garden soil in separate small flower-pots, and put them in the dark. After some time we select for the experiments proper five to ten seedlings very uniform in development, and with plumules about 2 cm. long. We place the pots in suitable heliotropic chambers, which have in the front wall a horizontal slit some centimetres in width. Owing to their vigorous circumnutation the plants grown in the dark are often not perfectly straight. We put the pots into the heliotropic chambers in such a way that none of the seedlings are inclined towards the light from the window. It can be made out that the heliotropic nutation begins at the extreme tip of the sheathing leaf of the plumule, and gradually proceeds downwards, while a greater and greater length of the upper portion of the leaf, which continues to incline forward, becomes straight. At last the curvature, generally a very sharp one, is found at the base of the sheath leaf, and the upper part of the leaf forms an angle of 60°–90° with the base of the leaf.*† This is the case at the end of

* The magnitude of the curvature can be determined by measuring. The measurements, which consist in determining the inclination of the inclined

some five to eight hours. If now, simultaneously with the objects mentioned, we expose seedlings of Avena to the light, after darkening a length of say 3 mm. at the tip of their plumules by covering with small caps of tinfoil (prepared by wrapping strips of tinfoil round a wire of suitable thickness, and carefully nipping the end of the tube so formed), then the nutation begins at the summit of the unshaded part of the leaf, it proceeds downwards more slowly than when the whole seedling is illuminated, and remains also comparatively flat, 10°–40°. The lower parts of the leaf are certainly in themselves heliotropically sensitive, but their sensitiveness as compared with that of the tip of the leaf is small. When the tip of the leaf is struck by the light, and gives a vigorous heliotropic reaction, the stimulus is transmitted to the lower part of the leaf, and induces there a far stronger nutation than would result from its own sensitiveness alone. Darwin (*Power of Movement in Plants*) also called attention to these interesting relations.

The transmission of stimulus from the tip to the base of the sheath leaves can also be demonstrated in the following manner. We cultivate oat seedlings in pots not quite filled with soil. When the plumule has attained a length of about 1·5 cm., we cover the seedlings with finely riddled dry soil, so that they only project for a length of 3 mm. at the tip. The soil is perfectly impermeable to light beyond a depth of 2–3 mm., and yet when the seedlings are illuminated unilaterally the basal part of the plumule curves owing to heliotropic stimulus conveyed to it from the tip. In this experiment the heliotropic curvature of the shaded part of the seedling is induced by the conveyed stimulus alone; under normal conditions the nutation of the basal parts of the sheath leaf is the combined result of this propagated stimulus and the direct heliotropic perceptivity of the organ. These experimental results are undoubtedly of great importance in interpreting heliotropic phenomena in general. The results of the following experiments are not less deserving of consideration.

The plumule of seedlings of Avena sativa about 1·5 cm. in

portion to the vertical, are made by means of quadrants, on which radii are drawn at intervals of 5°. The mean of several readings must always be taken.

† That at the close of heliotropic nutations the greatest curvature is found in most cases just at the base of the growing region, and not by any means at any particular time in the zone of strongest growth, is quite in harmony with the statements of Sachs (*Flora*, 1873, and *Lectures*) respecting the geotropic nutations of shoots. See also Müller-Thurgau, *Flora*, 1876, p. 90.

length is divided by ink-lines into zones of 2 mm. length. Measurements of the growth made in the usual way show that the tip of the plumule only grows very slowly; the rate of growth increases as we proceed downwards, attains its maximum say in the fourth or fifth zone, and then again falls off. Hence it is clear, and this is undoubtedly interesting, that the zone of greatest heliotropic perceptivity (the tip) is by no means at the same time that of greatest growth also.

If perfectly straight seedlings of Avena, grown in the dark, are decapitated by removing from their tip with a sharp transverse cut a zone 3 or 4 mm. long, and are then illuminated unilaterally, their growth is only slow, and heliotropic nutations are not performed at all. After a few hours, however, the rate of growth of the sheath leaf again becomes more rapid, and heliotropic sensitiveness also returns.* Many of the experiments described are also of great significance, because they teach that the power which the organs possess of reacting and their power of perceiving (their irritability and sensitiveness respectively) are to be regarded as two distinct things.

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[1] See Müller-Thurgau, *Flora*, 1876.

[2] See Oltmanns, *Flora*, 1892, p. 223.

[3] See Rothert, *Ber. d. Deutschen botan. Gesellsch.*, Bd. 10, and Cohn's *Beiträge zur Biologie d. Pflanzen*, Bd. 7. This latter research I have unfortunately been unable to consider further.

180. The Hydrotropism of Roots.

When the moisture in the medium in which roots are developing is not uniformly distributed the roots execute hydrotropic curvatures, and following Sachs' method[1] it can readily be determined that roots are positively hydrotropic. The apparatus necessary for demonstrating the phenomena here under consideration is shown in section in Fig. 160. A hoop of strong sheet zinc about 5 cm. high and 20 cm. in diameter is covered with wide-meshed netting, so as to form a sieve, of which the porous bottom

* The decapitation at first completely destroys the heliotropic perceptivity of the seedlings; they consequently do not curve, although growth is slowly proceeding. Later on a fresh "physiological apex" is to some extent developed. The seedlings are then almost as irritable as absolutely uninjured ones.

consists of the netting. We fill the sieve with moist sawdust, and lay in it soaked seeds. I experimented with Phaseolus seeds; we may, however, equally well employ other seeds (Pisum, Zea, etc.). The apparatus is then hung obliquely, by means of three threads, in a cupboard or under a large cardboard box, the base of the sieve being inclined at an angle of about 45° with the horizontal. The main roots of the seedlings, which develop in complete darkness, soon pass out through the meshes of the netting; they do not, however, grow straight downwards, but their tips at once

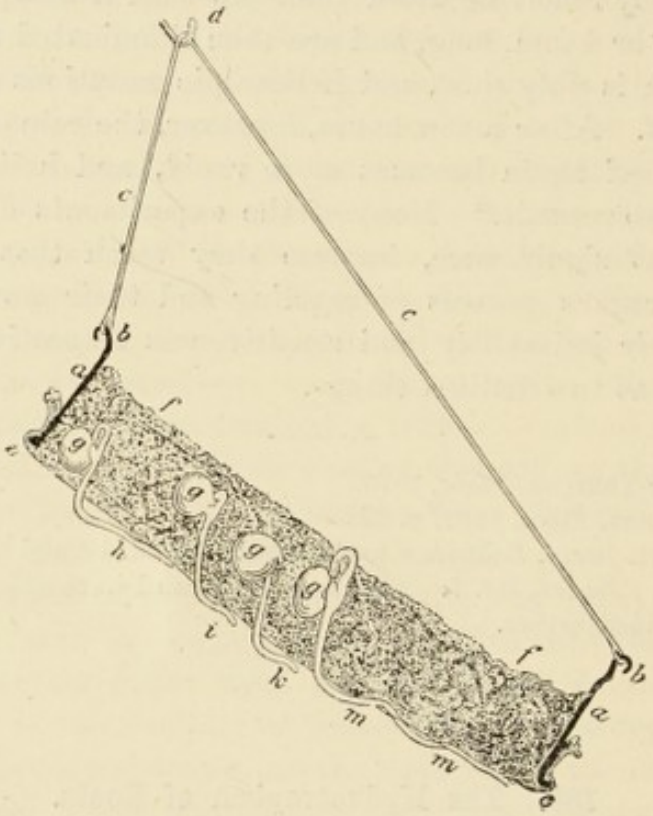

Fig. 160.—Apparatus for observations on hydrotropic root curvatures. (After Sachs.)

apply themselves obliquely to the under surface of the netting, and now continue their downward growth closely pressed to it. The roots on emergence turn, as our illustration also shows, towards the side on which the seed-bed makes the smallest angle with the vertical. The curvatures are due to a difference in the distribution of moisture on the sides of the roots turned towards and away from the seed-bed, though it seems specially noteworthy that just that side of the root becomes convex, and therefore grows most vigorously, which is not directed towards the moist base of the sieve. The root curvatures referred to completely

cease when the apparatus is suspended horizontally, or even obliquely in an atmosphere completely saturated with aqueous vapour, *e.g.* under a large bell-glass wet on the inside. In this case the main roots of the seedlings grow straight downwards. Growth curvatures due to non-uniform distribution of moisture cannot here take place; the roots growing in absence of light, in a space saturated with moisture, only answer to the directive influence of gravity.

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[1] See Sachs, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 209.

181. The Hydrotropism of Mucor Mucedo.

We have already made ourselves acquainted with Mucor Mucedo in 36. The sporangiophores of Mucor are distinguished, as Wortmann[1] determined, by being negatively hydrotropic. Hence, if they are growing in the neighbourhood of a moist body, they curve away from it. I have satisfied myself that this can easily be determined as follows.

Some cow-dung or horse-dung is left under a bell-glass for a few days. There develops a luxuriant growth of Mucor Mucedo, the fungus which we require for our experiment. We lay a small cube of bread, saturated with water, in a flat glass dish, and cover the dish with a sheet of glass, which fits closely to its edge, and is provided in the middle with a hole a few mm. wide. Before covering with the sheet of glass, we transfer to the bread, by means of a needle which has been sterilised by heating, some ripe sporangia from the Mucor growth on the dung, and distribute the spores on it. The spores quickly germinate; after one or two days sporangiophores are already growing out through the hole in the sheet of glass, and it is now our object to prove that these organs are negatively hydrotropic. For this purpose a strip of thick cardboard is fastened on a cork by means of shellac. We then saturate the cardboard with water, and place it in the immediate neighbourhood of the sporangiophores growing through the hole in the glass. The whole is now covered with a cardboard box in order to exclude the light, as also earlier in the course of the experiment, viz. after the sowing of the spores. After about twenty-four hours, the sporangiophores will have increased considerably in length, and it is easy to see that they have not grown

vertically upright. They are curved; the convex side is directed towards the moistened strip of paper.

If we put not a wet but a dry strip of cardboard in the neighbourhood of the sporangiophores, they do not curve. The nutation, therefore, is not, as supposed by van Tieghem,[2] to be regarded as somatotropic, *i.e.* as due to the mass of the paper, but actually as hydrotropic.

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[1] See Wortmann, *Botan. Zeitung*, 1881.

[2] See van Tieghem, *Extrait du Bulletin de la Société botan. de France*, T xxiii.

182. Thermotropism.

It is of interest to satisfy oneself that growing plant structures perform irritation movements when their opposite sides are exposed to different temperatures.[1] We make the experiments in a large room, with a north aspect if possible, and subject to the smallest possible variations in temperature. At one end of a long working bench a plate of sheet-iron, previously smoked, and about 60–70 cm. square, is set up on a strong support with its surface directed vertically to the window panes. The back of the plate can be warmed by means of four gas flames, the position of which can be adjusted according to requirements. The objects to be examined (seedlings of Lepidium and Zea grown in sawdust in flower-pots) can now be placed at various distances from the plate. To eliminate heliotropic nutations a large plane mirror is set up behind the originally perfectly straight seedlings, and parallel with the window, through which softened light enters through curtains. Before placing the pots in position, we warm the plate, and suspend thermometers at different distances from it. When these indicate the particular temperatures at which we desire to experiment, the seedlings are brought into their immediate neighbourhood, and the experiment begins. Experiment with Lepidium. Ten seedlings. Height 3–4 cm. Temperature of room 12° C. Temperature over the middle of the flower-pot 30–35° C. After a few hours marked negatively thermotropic nutation. Experiment with Zea. Two seedlings, 2–3 cm. high. Room temperature 12° C. Temperature over middle of flower-pot 30° C. After a few hours positively thermotropic nutation (Wortmann).

The temperature optimum for the growth of Lepidium lies

somewhere in the neighbourhood of 28° C., but that of Zea at 33° C. The origination of the curvatures can thus not be regarded as the result of difference in growth on the opposed sides of the seedlings, directly dependent on the temperatures obtaining on these two sides. If that were the case, then under the conditions stated the Lepidium seedlings, *e.g.*, must have curved straight towards the source of heat, since the side remote from the plate was exposed to a temperature more favourable for its growth than the side turned towards the plate. The curvatures are, on the contrary, to be regarded as irritation effects called forth by the heat rays. Very searching investigation of the subject, however, is still necessary.[2]

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[1] See Wortmann, *Botan. Zeitung*, 1883 and 1885.

[2] According to Jönsson (*Berichte d. Deutschen botan. Gesellschaft*, Bd. 1, p. 512), roots also exhibit rheotropic properties. My investigations on the subject are not yet complete. As regards the aerotropism (see Molisch, *Berichte d. Deutschen. botan. Gesellsch.*, Bd. 2, p. 160) and galvanotropism (see Elfving, *Botan. Zeitung*, 1882) of roots, I have as yet made no experiments.

183. Aerotropism and Chemotropism of Pollen Tubes and Fungal Hyphæ.

The germination of pollen grains, of which we shall frequently have to speak, takes place best in sugar solutions (cane-sugar) of particular concentration. The concentrations are first given in the following table for some kinds of pollen :—

Allium Victoriale	3 per cent.
Anthyllis vulneraria	15 ,,
Berberis vulgaris	20 ,,
Colchicum autumnale	40 ,,
Digitalis ambigua	10 ,,
Fritillaria imperialis	10 ,,
Narcissus poeticus	10 ,,
Vincetoxicum officinale	15 ,,

We now prepare sugar solutions with addition of 1–2 per cent. of gelatine. This solution sets at 18° C. to a jelly. A drop of the solution is placed on an object-glass, and treated with pollen grains, which we rapidly distribute very evenly by means of a needle. We now lay on a cover-glass, taking care in doing so that none of the fluid escapes beyond the edge of the cover-glass. The formation of bubbles is also to be avoided. The slide cultures are

placed at 18° C. under a bell-glass standing in water. If, *e.g.*, we experiment with pollen grains of Narcissus Tazetta (7 per cent. cane-sugar solution), we find that many grains have germinated even after six hours. But only the pollen grains near the edge of the cover-glass have developed tubes, the rest owing to want of Oxygen have not germinated. The tubes almost all grow towards the middle of the drop; they exhibit distinct negative aerotropism. It is however to be observed that there are many kinds of pollen whose tubes are in no way aerotropically irritable (Orobus vernus, etc.).

According to my experience the growing pollen tubes of species of Lathyrus are excellent objects for the determination of negatively aerotropic behaviour. The pollen grains are sown in sugar-gelatine, containing 15 per cent. of cane-sugar.

Many pollen tubes (Narcissus Tazetta, N. poeticus, Fritillaria imperialis, Vincetoxicum officinale, etc.) also exhibit very vigorous chemotropic irritability.

We distribute the pollen grains in the sugar-gelatine drop, and lay in the middle of this a fragment of freshly cut stigma or style tissue or ovules of the corresponding plants. The grains germinate, and the tubes developing in the neighbourhood of the fragment of stigma, etc., being positively chemotropic, turn towards them. If we kill the fragments of stigma, etc., by dipping into hot water before laying them on the drops of gelatine, the pollen tubes behave exactly in the same way. This proves that the direction of growth observed in the tubes in the case before us cannot be due to their aerotropic irritability, since the masses of tissue when dead do not, as when living they of course do, produce Carbon dioxide, which might influence the distribution of Oxygen tension in the preparation.

Frequently in investigating chemotropism in pollen tubes, it is well to put them into a drop of gelatine containing only small quantities of sugar (*e.g.* ·2–·4 per cent.), then lay on the fragment of stigma, etc., and put the preparation in a moist place.

Cane-sugar, which occurs in the cells of the gynæceum, and is secreted by its cells, is one of the most effective stimulants for the tubes.

We inject leaves of a Tradescantia (*e.g.* T. discolor) under the air-pump with cane-sugar solution of certain strength. The now translucent leaves are rapidly rinsed with water, and dried externally with blotting-paper. We now powder pollen on the

under side of the leaves, which are well supplied with stomata, and bring them into a moist space. After a shorter or longer time we determine that the pollen tubes in their growth show an inclination to direct themselves towards the stomata, and enter them. If the leaf has only been injected with water, the tubes grow over the surface indifferently in all directions. Cane-sugar is therefore a body which incites the pollen tubes to chemotropic movements.

The germinal hyphæ of fungi are also chemotropically irritable. If Tradescantia leaves are injected with 2 per cent. cane-sugar or meat extract, and Mucor spores are then germinated in the moist space on their under surfaces, we find here also that the tubes turn towards the stomata, and enter them. If the concentration of the cane-sugar solution is reduced to 0·1 per cent., the tubes only give a very feeble positive reaction; the same is the case when the concentration of the sugar solution is considerable (*e.g.* 20 per cent.).[1]

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[1] See Molisch, *Sitzungsber. d. Akad. d. Wiss. zu Wien*, Bd. 102, Abth. 1, July, 1893; Miyoshi, *Botan. Zeitung*, 1895, and *Flora*, 1894.

184. Movements of Foliage Leaves and Floral Structures Induced by Changes in Illumination and Variations of Temperature (Nyctitropic Movements).

There exist many foliage leaves and flower leaves which, as the experimental researches of several physiologists have shown, react by growth movements only to an insignificant extent in response to variations of temperature, but conspicuously in response to changes in illumination. We will first endeavour to make out some of the phenomena in question.

We make our observations on plants of Impatiens parviflora, Chenopodium bonus Henricus, and Mirabilis jalapa, growing in the open. The movements which interest us are clearly exhibited in the younger, not yet fully developed foliage leaves. In the daytime, these leaves are disposed more or less horizontally; at night they assume another position. In Chenopodium, and especially in Mirabilis, the leaves raise themselves in the evening, while in Impatiens they sink at this time, and hence as darkness comes on they pass from a horizontal to a vertical position. On the following day the leaves return again to their light position.

If we observe seedlings of Raphanus sativus grown in flower-pots, it is seen that their cotyledons are expanded in the daytime, but at night appear more or less closed up. Young leaves of Tropæolum majus illuminated from above assume in the daytime a position such that the incident rays of light strike them at right angles. At night they place themselves vertically. According, however, to the results of my investigations with pot plants, this only takes place fully at a comparatively high temperature.

Concerning the growth movements of flowers induced by change of illumination, and using mostly plants growing in the open, I made the following observations which we may repeat.

FIG. 161.—Flower scape of Leontodon hastilis; inflorescence closed.

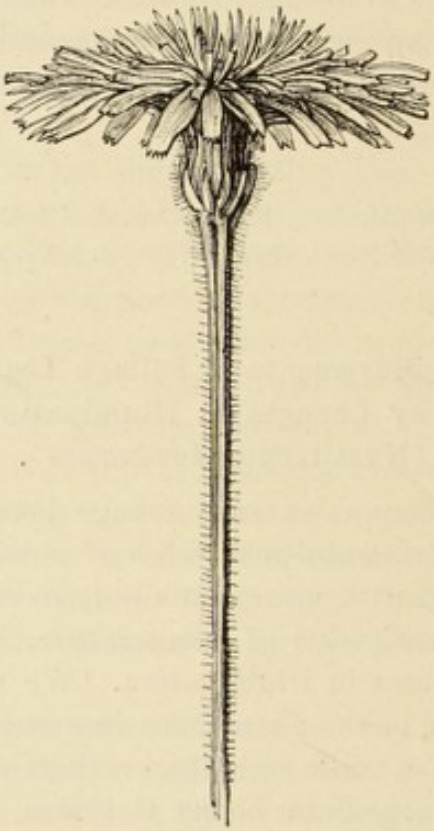

FIG. 162.—Flower scape of Leontodon hastilis; inflorescence expanded.

I found that the flower heads of most specimens of Taraxacum officinale, on sunny days at the beginning of May, open between 7 and 8 o'clock in the morning, and close again between 4 and 5 o'clock in the afternoon. The flowers of Tradescantia remain open all day if the sky is overclouded; under a cloudless sky,

however, they close in the morning at 10 o'clock. The flower heads of Tragopogon pratensis are open in the morning. In June, however, they close in sunshine at 9 o'clock, and with a clouded sky at about 11 o'clock. The flowers of Adonis vernalis, and the flower heads of Bellis perennis and Leontodon hastilis close in the evening and open the next morning (see Figs. 161 and 162, in which are represented scapes of Leontodon in one case with a closed, in the other with an open flower head). I found that the flowers of Adonis, at the end of April, closed at 3 o'clock in the afternoon, while flower heads of Bellis plants just near them did not close till an hour and a half later. The flowers of Œnothera biennis expand in the evening and close in the morning. I cut scapes of Leontodon hastilis, and put them in the dark in the daytime with their stalks in water. The flower heads closed in the evening, but opened on the following day, although darkened all the time, and in the evening once more exhibited closing movements, though not, it is true, very vigorous ones. The next day the scapes were again exposed to the daylight. In the evening of that day, the flower heads closed in a perfectly normal manner. These experiments teach that the individual flowers of the flower heads of Leontodon hastilis—and many other flowers behave in an analogous manner—when exposed to continuous darkness, exhibit after-effect movements, which cause the flower heads to open in the daytime, and close at night. It is true these after-effect movements do not continue long. The flowers become after a time motionless (darkness-rigor), but can be rendered phototonic again by renewed illumination.

From what has been said, it will now be clear that the periodic movements of foliage leaves and flowers taking place under normal conditions, *i.e.* with alternation of day and night, are not the immediate result of the alternation of illumination alone. The daily period is rather the resultant of paratonic effects due to the alternating conditions of illumination, and after-effect movements which, however, also have their ultimate origin in the alternation of day and night. This also renders intelligible a fact of the accuracy of which I have satisfied myself, that, *e.g.*, flower heads of Leontodon hastilis placed in the dark in the forenoon, close only a little earlier in the evening than others which have been illuminated during the day. The paratonic effect of the darkening is certainly to be recognised here, but the flower heads, owing to after-effects, do not close until the darkening has continued for

some time. In other cases, however, the paratonic effects may be much more obvious.

The movements of foliage and flower leaves induced by change in illumination, are due to processes of growth, as Pfeffer[1] showed with complete certainty. The change in illumination acts in the same sense on the growth of the basal parts of the foliar structures which cause the movements, but the opposed tissue complexes of the organs are not affected with the same rapidity, and hence the nyctitropic movements here under consideration are brought about. The biological significance of the vertical position assumed at night by many foliage leaves, is clearly to be sought in the fact that it prevents a too rapid radiation of heat, which might easily have disastrous consequences for the plants, while the opening and closing of flowers is related to conditions of pollination.

In various plant structures changes of temperature, like changes in illumination, induce active movements. We have here to do chiefly with flowers, and excellent objects for investigation are flowering specimens of Tulipa gesneriana, and above all of Crocus vernus (white flowered variety). I took pot plants of Crocus vernus from a room at a temperature of 19° C. into one at a temperature of 5° C. The flowers, at first open, soon closed. If Crocus plants with closed flowers were taken out of a room at a temperature of 5° C. into one at a temperature of 19° C., the flowers speedily opened. The closing movements of the flower leaves on cooling on the one hand, and their opening movements on warming on the other, proceeded so rapidly in my experiments, that their commencement could be seen with the unaided eye within a few minutes of the change of temperature. Crocus flowers react, moreover, to very slight variations of temperature. The movements induced by change of temperature take place in the light as well as in complete darkness.

That the above-mentioned movements induced by changes of temperature and illumination are the results of processes of growth, can only be determined by somewhat delicate measurements. We select Crocus for investigation. Here the zone in which especially the movement of the perianth leaves takes place, lies just above the perianth tube, in the lower fifth of the perianth segments. We cut off the three inner segments, and make fine ink-dots at intervals of about 3 mm. on the outside and inside of the outer segments, along the middle of the zone of greatest

power of movement. Easily recognisable points or angles of the ink-dots serve as points of reference for the measurements, which are made by means of the horizontal microscope described in 153. If, *e.g.*, dots are made on the perianth segments of the closed flowers, and we determine the distance between the marks again after the flower has been induced by rise of temperature to open, then we find hardly any alteration in the distance between the marks on the outside of the segment, while on the inside the distance between the marks has increased considerably, perhaps by 0·08 mm., if the original distance between them was 3 mm. The lengthening of the now convex side of the segments is permanent, and therefore fixed by growth. When the flowers close, the outer side, which becomes convex, lengthens much more conspicuously than the opposite side.

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[1] Pfeffer, *Pflanzenphysiologische Unters.*, 1873.

185. The Darwinian Curvature, etc.

We germinate seeds of Vicia Faba in sawdust, and leave the seedlings in the sawdust until the roots have attained a length of a few cm. It is now required to injure the tip of the root on one side, and, according to my experience, this is most conveniently effected by touching the root on one side with a small fragment of lunar caustic (Silver nitrate). Care must be taken that only a short length of the root tip, say 1·5 mm., is brought into contact with the lunar caustic. From what was said in 157, it appears not immaterial which side of the root receives the stimulus, and it is best to stimulate neither the anterior nor the posterior surface of the root, but one of the flanks. The plants are now placed in glass cylinders as described in 154, and laid aside in the dark. After twelve to twenty-four hours we find that the side of the root touched with the caustic has become convex. On the side stimulated, the growth, in the region of the root capable of growth, is considerably more rapid than on the opposite side, so that unilateral injury to the root tip has induced a characteristic nutation, which we term the "Darwinian curvature." Nutations likewise take place if the injury to the tip of the root is effected not by means of Silver nitrate, but in some other manner; it is, however,

of biological significance that the roots always turn away from the bodies causing the injury, and acting as stimuli.[1]

In contrast with the above, we have root nutations brought about by stimulation not of the root tip, but of the region of the root which is in active growth. The roots then bend, as is the case with tendrils, towards the bodies producing the stimulus. A few pea seedlings, with roots about 2 cm. long, are fixed on pins, and arranged under a bell-glass standing in water, with their roots directed horizontally. Beside each seedling we bring a thin pin, so as to touch the root in its most actively growing region (*i.e.* about 3 mm. from the tip). The contact acts, as in tendrils, as a stimulus, and since the free side of the root becomes by more active growth convex, the root curves after a time towards the pin.

Characteristic stimulation effects are brought about, as may here be briefly mentioned, by the bite of insects. The numberless varieties of galls occurring on leaves are produced in this way, and it is hence not without interest, from the physiological point of view, to examine a gall carefully.

On the leaves of many kinds of willow we often find during the summer galls resembling a small bean, fleshy, and projecting on both sides of the leaf. These are due to the bite of leaf wasps (Nematus Vallisnerii). The wasps lay their eggs in the tissue of the leaf while it is quite young, and the grub, creeping out of the egg, develops in the gall, which attains a comparatively large size. The stimulus produced by the bite of the insect results in hypertrophy of the leaf tissue, which expresses itself in the formation of the gall. Microscopic examination of thin transverse sections teaches that the Nematus galls consist chiefly of small-celled tissue, the elements of which are approximately isodiametric. In this tissue are enclosed in various places cells elongated in a radial direction. It is also to be remarked that the tissue in the middle of the gall is made up of smaller cells than that at the periphery.

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[1] See Darwin, *The Power of Movement in Plants*, and Wiesner, *Bewegungsvermögen der Pflanzen*, p. 139.

III. THE WINDING OF TENDRILS, AND TWINING PLANTS.

186. Generalities Respecting the Winding of Twining Plants.

If we examine a hop stem wound round a support, we shall find that the spiral always runs from right below to left above. The hop is a typical right-winding plant.* It may here also be remarked that both stems and leaf-stalks of hops are provided with peculiarly formed appendages, which function as clinging or clasping organs, since they assist in fixing the plants. If we remove, *e.g.*, from the leafstalk of Humulus, small strips of epidermis, and examine them microscopically, it will be seen that on the surface are seated wart-shaped emergences. Each of these bears at its end an anchor-shaped hair, which forms an excellent organ of attachment.

A few flower-pots, not too small, are filled with well-watered garden soil, and soaked seeds of Phaseolus multiflorus are laid in them. It is advisable to put several seeds into each flower-pot, and then later the weakly plants may be removed, and only one vigorous specimen in each pot be further cultivated. It is best to grow the plants in a place where they will be exposed only for a short time in the day to direct sunlight, being for the rest of the day exposed to bright diffuse daylight. When the third internode begins to develop, we place long sticks close to the plants. These wind

* In botany it is customary to associate with the terms "right" and "left," as applied to spirals, a meaning opposite to that which they have in mechanics.

FIG. 163.—Winding shoot of Phaseolus multiflorus.

round the sticks, but the bean is a left-winding plant, not like the hop a right-winding one. The spiral extends from left below to right above. Fig. 163 clearly shows this; and it indicates further, what we shall consider carefully in 189, that the older turns of the spiral are tighter and steeper than the younger ones. This is seen with especial clearness when the supports used are thin, not exceeding, in experiments with Phaseolus—for example, a thickness of a few millimetres. It is not uninteresting to observe a number of twining plants, which have been allowed to wind round supports of different thicknesses. I let bean stems wind round stretched thread, wires 1 mm. in diameter, and sticks 4, 16 and even 30 mm. in diameter.

187. Rotating Nutation.

In carefully studying the winding of twining plants, it is of the utmost importance to make ourselves acquainted with the rotating nutation performed by shoots capable of twining. We lay seeds of Phaseolus multiflorus in garden soil contained in fairly large flower-pots. The first internodes of the young plants do not exhibit the phenomenon of rotating nutation, or not clearly, but the following ones do. The long terminal bud nods sideways, owing to its own weight, and if we observe it accurately it will be found that it is not at rest, but in a state of uninterrupted movement, by which it is carried round in a circle.

To study more closely the remarkable phenomenon of rotating nutation, we employ other plants besides those of Phaseolus, *e.g.* shoots of Calystegia 30 cm. in length, or the shoots of other twining plants. It is very important, however, for the plants to be in a state of active growth, and exposed to very favourable conditions. The basal part of the shoot is erect, but the summit of the shoot is bent over in a wide arc, so that the apex is horizontal or even directed a little downwards. We now make an ink-line along the convex side of the shoot, running parallel with its axis, and place the shoot with its convex side towards us (see Wortmann, *Botan. Zeitung*, 1886). The plane of curvature is then vertical to the plane of our body, the apex of the shoot is turned away from us, and points, say, to the east. If we look at the shoot again, after half an hour or three-quarters of an hour, we perceive striking changes in it. The horizontally hanging apex points to

the north, the plane of curvature is as before vertical, but parallel with the plane of our body, and the ink-line is no longer on the convex side, but on the left flank of the shoot. It is thus shown that the zone of most active growth, indicated by the convex side of the shoot, has in our experiment moved 90° to the right. The apex of the stem, however, has in the process described a horizontal arc of 90° to the left. The further behaviour of the shoot is easy to follow. After a further interval of half an hour or three-quarters of an hour the ink-line is found on the concave side of the shoot, and the original position is not again attained until two or three hours from the start. Then begins a fresh revolution. It is further noteworthy that the zone of most vigorous growth almost always runs along the upper side of the curved part of the shoot, which results in the plane of curvature being always vertical. The revolving motion of the end bud from what we have seen is due to the fact that the zone of most vigorous growth moves round and round the stem.

It must now be emphasised that the rate at which a complete revolution of the apex of the shoot is executed is essentially dependent on external factors, particularly on temperature. If nutating shoots are exposed to unilateral illumination, the movement towards the light is more rapidly effected owing to heliotropic processes than the movement away from the source of light. Phaseolus and Calystegia shoots exhibit very active movements, other twining plants only slow ones, but even in one and the same object, and with approximately constant external conditions, the rate of movement may vary in course of time to a notable extent. Variations from the normal behaviour of the shoot also occur in consequence of which the plane of curvature is not always vertical, *i.e.* the zone of vigorous growth does not always lie along the upper side of the curvature, but is displaced. Different parts of the rotating stem may, moreover, be curved in different planes. Attention must be paid to all these points, and it is well to observe a number of plants continuously, and accurately note the results obtained.

To obtain a further insight into the highly complicated phenomena here under consideration, it is necessary first to make the following experiment. Phaseolus plants or Calystegia shoots at a stage of development in which the rotating nutation is just about to begin, are slowly rotated on the clinostat in the horizontal plane, so as to exclude heliotropic nutations. We may also observe

shoots already circumnutating, in that case merely binding the lower parts of the shoot to a stick, so that the end of the shoot to a length of 10–15 cm. is left free and quite vertical. In each case we shall perceive sooner or later, how the free shoot end for a length of a few cm. begins to execute a generally very sharp curvature. This so-called preliminary curvature [*Vorkrümmung*] is not caused simply by the weight of the terminal bud, as follows from the fact that it persists if we place the shoot in another position.

The following is also important. Shoots capable of rotating nutation are like other shoots geotropically irritable in their growing region. If, *e.g.*, we stick shoot ends of twining plants into moist sand as indicated in 173, their negative geotropism is clearly exhibited. The zone of greatest irritability is always situated a few cm., *e.g.* 10 cm., distant from the apex of the shoot, as can easily be made out by using portions of different lengths.

These facts were first established in detail, by Baranetzki [1] and Wortmann.[2] They both showed also that the rotating nutation itself is not, as was before supposed, purely spontaneous, but is the resultant of two forms of movement. The shoots of twining plants are of course capable of spontaneous movements, and in consequence of this they perform the so-called "flank-curvatures" to which also the above-mentioned preliminary curvature owes its origin. This flank-curvature, to be designated as the horizontal component of the rotating movement, is distinguished by the fact that always one flank of the shoot—thus, *e.g.*, in left-winding plants the right—grows more vigorously than all the rest, and hence, if it alone were operative, would result in a spiral coiling of the shoot ends. With the horizontal component, however, is associated another acting vertically, viz., negative geotropism. As was emphasised, and as is very important, it does not act on all zones of the shoot with equal energy, and leads further to the result that the convexity of the curvature of rotating shoots is always normally on their upper side. Hence is brought about a displacement of the flanks and the commencement of circumnutation. (See Wortmann, *Bot. Zeitung*, 1886, pp. 638–642.)

We cannot here pursue further Wortmann's theoretical considerations, there and elsewhere expressed. Essentially I consider them sound.

It is now of special importance, with a view to proving that

rotating nutation is a resultant movement brought about by the co-operation of two factors (spontaneous nutation and negative geotropism), to experiment with the help of the clinostat. The apparatus is set up on a vibration-free table, and we work with pot plants of Phaseolus or plants of Ipomœa chrysantha raised from seed. We fix the lower part of the plant, which has so far been free from support, to a suitable stick standing in the soil of the culture vessel, so that only the summit of the shoot to a length of a few centimetres remains free. This shoot end must be very vigorous in development, and must not bend much by its own weight when the plant is placed in the horizontal position. In this horizontal position a flank-curvature (preliminary curvature) now very soon appears in the originally straight shoot end. If the plant is now transferred to the clinostat, and set in slow rotation in the vertical plane (it is sufficient if the clinostat is so adjusted that one complete revolution is effected in about ten minutes, see 176), the preliminary curvature soon disappears again, being compensated by processes of growth, and the shoot becomes straight. New nutations, however, arise, which are again compensated or remain more or less persistent. The shoot has therefore an internal tendency to movements of nutation. When rotating on the clinostat, however, since the effect of gravity is eliminated, rotating nutation cannot take place, but only an undulating nutation. The power of the shoot to perform spontaneous nutations is a necessary prior condition for the production of flank-curvatures, which as we saw act as horizontal components in rotating nutation. This latter is therefore the resultant of two factors—of the spontaneous nutation of the shoots, and of their negative geotropic movements.

The clinostat experiments are to be continued, if possible, for a good time, so that we may satisfy ourselves by accurate observation of the behaviour of the plants that rotating nutation does not take place. Then it is convenient to use for the support a glass tube about 1 cm. in diameter, standing in the soil of the culture vessel, and slip into it a number of successively smaller glass tubes. These we draw out as the shoot grows, in order that with increasing length it may always be conveniently fixed, while only its actively growing terminal portion is ever free.

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[1] Baranetzki, *Mémoires de l'acad. impér. de St. Pétersbourg*, Sér. vii., T. 31, No. 8, 1883.

[2] Wortmann, *Botan. Zeitung*, 1886.

188. Free Winding.

From plants of Ipomœa purpurea or Phaseolus growing in the open, we cut very vigorous shoot ends about 20–30 cm. long, as straight as possible, and not yet grasping any support. These are now placed with their lower ends in a small glass vessel, filled with water, and covered with a large bell-glass or put into a large glass cylinder, whose mouth is covered with a sheet of cardboard. In order to keep the air surrounding the objects very moist, we wet the sides of the bell-glass or glass cylinder with water. Under these conditions the shoots continue their growth, and after two or three days have formed a number of free turns.[1] I have obtained this result in experiments with shoot ends of Phaseolus and Ipomœa purpurea. The latter plant serves particularly well, and I saw free turns originate whether the plants were left in the dark or were exposed to diffuse daylight. The drawing at the side (Fig. 164) represents a shoot of Ipomœa which has formed free turns. It is shown, and I observed this still more clearly in other cases, that the lower and therefore older parts of the coiled stem are directed more steeply than the younger parts, a fact to which we shall return later.

Fig. 164.—Shoot of Ipomœa purpurea, with free turns.

[1] See Sachs, *Lectures on Plant Physiology*.

189. The Mechanics of the Winding of Twining Plants.

If the end of the stem of a twining plant is being carried round in space by rotating nutation, it is obvious that it may easily encounter a suitable support.

To understand the phenomena which are to be observed in twining, it is of the first importance to consider the rotating nutation, in the causation of which, as we saw in 187, the persistent negatively geotropic behaviour of the stem plays so im-

portant a part, and also the resistance of the supports. These two factors cause winding stems to run round the supports in a spiral line, and they also afford us an explanation of the fact which we observed, that the uppermost turns made round a support are flat and relatively wide, while the lower ones are steeper. We must bear in mind, viz., that the stems of twining plants continue to grow for some time after coiling, and consequently they become elevated under the influence of gravity. If a support is present, the older parts of the stem cannot completely straighten themselves, because of course the support always stands in the way. The winding stems now closely apply themselves to the supports in a spiral line, and the ultimate angle of inclination of the shoot axis will be the less, the thinner the support. With thick supports, the application of the older parts of the stem to the supports takes place early; the erection of the internodes is soon arrested, and the completed spirals therefore appear comparatively flat.

It is perhaps at first sight surprising that free turns only seldom appear in a typical form in the overhanging shoot ends of twining plants growing under perfectly normal conditions in the open, whereas they readily develop, as we have seen, in pieces of stem cut off. The case soon becomes clear, however, on careful consideration. The vigour of growth in cut shoots is at any rate considerably reduced. Free turns can, it is true, be produced, owing to the rotating nutation associated with the growth, but the geotropic elevation of the internodes is only imperfectly exhibited. The shoot ends of twining plants vigorously growing in the open and projecting beyond the supports, react for the most part so well to the action of gravity, that usually their internodes straighten out almost completely, and consequently no permanent free turns can be developed.

We now proceed to various experiments, the results of which are calculated to afford us a deeper insight into the mechanics of the winding of twining plants.

Beside a young, very vigorous plant of Phaseolus multiflorus, grown either in a flower-pot or in the ground, is placed a support 30 mm. in diameter. After the stem has wound round it a few times, the support is rapidly removed, and replaced by a thin one, only a few millimetres in diameter. The turns of the stem, naturally, are not at first applied to this thin support. We now observe that the upper advancing end of the stem forms new

turns, but for us it is of special importance that the younger of the turns which were formed round the thick support, become steeper in the course of one to two days, and apply themselves closely to the thin support. This is due to the continued growth of the parts in question, and their consequent geotropic elevation. The older of the turns formed round the thick support on the contrary remain comparatively flat; they undergo no further changes since the growth of the older internodes has already ceased.

A vigorous pot plant of Phaseolus, which has made a few turns round a support, is placed upside down, some strips of wood being placed across the flower-pot to prevent the soil from falling out. The younger, still vigorously growing parts of the stem soon begin to unwind from the support, and the end of the stem directs itself upwards. This result only becomes intelligible when we reflect that in every transverse zone of the stem still in a state of growth, there is always, owing to the influence of the rotating nutation, a tendency to keep growing in the direction of a left-handed ascending spiral. After the inversion of the bean plant, the concave side of the stem, turned towards the support, must therefore become convex, and this results in the unwinding of the parts of the internodes which are still growing.

That gravity is really of importance in the causation of rotating nutation and winding, has already been shown in 187. The following experiment teaches the same thing. The flower-pot in which a vigorous bean plant has developed and already made several turns round a support, is fixed on the clinostat, and slowly rotated in the vertical plane. The rotation is made in a direction opposite to that of the normal winding. We now see that the parts of the stem still capable of growth loosen themselves from the support. The youngest turns unwind, and the shoot stretches itself more or less straight. Here the coiled, still growing part of the shoot, on suspension of the action of gravity, behaves on the clinostat exactly like, *e.g.*, a simply geotropically curved shoot. Such a shoot, if capable of growth, straightens under the conditions described; so also a coiled shoot, whose power of winding is likewise dependent on the co-operation of geotropism. The straightening is the result of internal growth determinants which are very generally operative when plant structures which exhibit nutations are placed under conditions in which the curvature determinant does not act. This power of plant structures to compensate

curvatures Vöchting termed rectipetality (*Bewegungen der Blüthen und Früchte*, p. 31), without however thereby professing to make clear the nature of the phenomenon.

It must also be regarded as a consequence of the co-operation of geotropism in the winding of twining plants, that they cannot coil round horizontal supports, a fact of which we can easily satisfy ourselves by experiment. Bean plants will not wind round supports inclined more than 40°.

The following experiment is also very instructive, showing among other things that the formation of turns in twining plants is quite certainly not the result of contact stimulus. On the end bud of a young pot plant of Phaseolus which has already coiled a few internodes round a support, is fastened a fine thread which runs over a lightly running pulley, brought vertically over the plant. On the free end of the thread we hang a small weight (in my experiments, I used a weight of 1 gr.) just sufficient to support the stem. After one to two days, the terminal portion of the stem will have formed a number of free turns (see Fig. 165), which however, as the apical growth of the stem continues, gradually disappear again, since geotropism, as under other conditions, ultimately causes the internodes to straighten out. If before beginning the experiment, we make fine ink-dots along the stem at short distances from each other, it will be found, after the disappearance of the free turns formed in the first place, that the dots are no longer in a straight line, but are arranged on a spiral ascending from the left. Homodromous

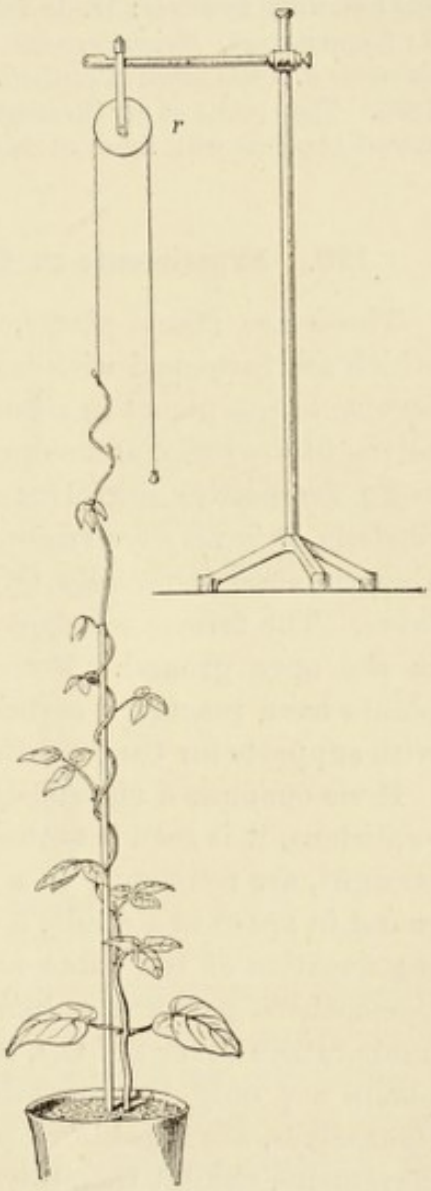

Fig. 165.—Phaseolus plant, whose stem above the support has made free turns.

torsion has therefore developed, as may very frequently be observed in twining stems. The origin of this torsion is most intimately bound up with the development of the free turns of our stem. When the turns by straightening of the stem disappear, they are converted into a homodromous torsion, *i.e.* a torsion corresponding in direction with the winding of the plant.[1]

[1] The more recent researches on twining plants will be found especially in the following treatises: H. de Vries, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1; Schwendener, *Monatsberichte d. Berliner Akad.*, 1881, December; Ambronn, *Berichte d. Sächsischen Gesellsch. d. Wissensch.*; Wortmann, *Botan. Zeitung*, 1886. The results of my investigations respecting twining plants agree in all essential points with those of Sachs and Wortmann.

190. Experiments on the Tendrils of the Cucurbitaceæ.

There are many plants, belonging to very different families, which are furnished with tendrils. These thread-like organs are of service to the plants in climbing. They are able to attach themselves to supports, and so prevent the plants from falling.

To familiarise ourselves with the remarkable peculiarities of tendrils, it is very convenient to employ for examination a series of Cucurbitaceæ, especially Cyclanthera explodens and Sicyos angulatus. The former we grow in not too small flower-pots, the latter in the open ground. Both are raised from seeds.* When the plants have reached a certain size, it is necessary to furnish them with supports for the tendrils to fix themselves to.

If we examine a vigorously developed specimen of Cyclanthera explodens, it is seen that the tendrils not yet fixed to supports, and straight, are constantly in a state of movement. They are carried round in space in a circle, a fact which is really due to the rotating nutation of the shoot carrying them. I observed a tendril of Cyclanthera perform a complete revolution, at a high summer temperature (over 20° C.), in the course of an hour. But in many plants not only the tendril-bearing shoots, but also the tendrils themselves, are capable of performing rotating nutation.[1] These movements exhibit themselves in a pure form when we exclude the shoot nutations by fixing the shoots to a stick at the point of insertion of the tendrils. The biological importance of the nutations is very considerable. The growing tendrils are thereby carried

* The Sicyos plants are first cultivated in flower-pots, and then, when they have attained a sufficient size, transplanted.

round in space, and are more likely to encounter suitable supports, to which they can attach themselves.

If it is desired to investigate carefully the irritability of tendrils, Sicyos angulatus is specially to be recommended as research material, and I have carried out many experiments with it. When young, the separate branches of the tendrils of this plant are spirally coiled. When however they have extended, and while still actively growing, they become exceedingly sensitive. At a low temperature naturally the irritability is less than in very

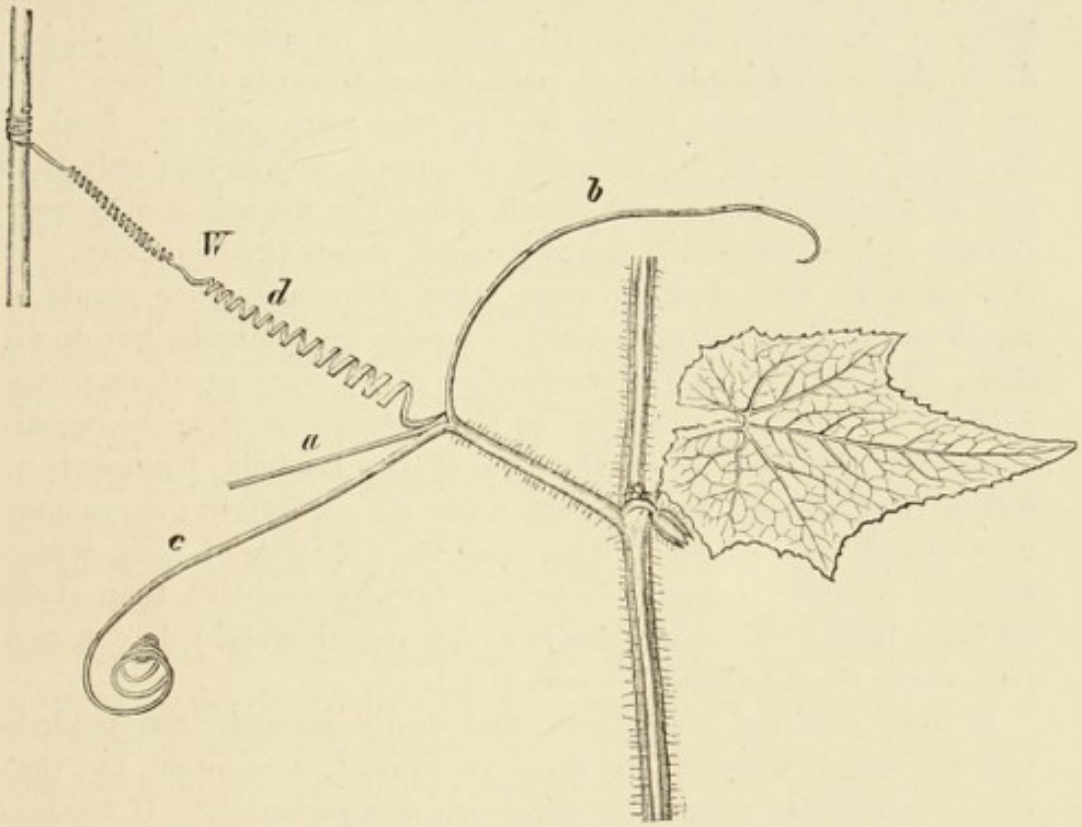

FIG. 166.—Portion of a shoot of Sicyos angulatus with a tendril.

warm weather. If, on a warm day, we carefully draw an extended tendril branch between the fingers, it at once curves considerably and the movement exhibited is so rapid that we can follow it directly with the eye. Fig. 166 shows part of a shoot of Sicyos angulatus bearing a tendril. One branch *a* is not stimulated, and so is still extended in a straight line. The branch *b* having been slightly stimulated by means of a thin wooden rod has performed an inconsiderable curvature, while the branch *c* having been strongly stimulated has performed a vigorous curvature. To the branch *d* we shall return later. If a tendril of Sicyos

is caused to curve by brief contact with a solid body, and then left to itself, it straightens again completely, and is once more sensitive to contact. At a high summer temperature, the straightening of the tendrils of Sicyos proceeds comparatively quickly, and even in experiments with Cyclanthera tendrils I found that a tendril strongly curved owing to stimulation, had, at a temperature of about 22° C., extended again in the course of an hour, and was once more sensitive to stimulation.

If a branch of a Sicyos tendril is touched at different points with a thin wooden rod, we shall easily make out that the irritability of the organ is especially great in its anterior third, but diminishes considerably as we pass thence towards the base. We can further determine that in the sensitive zone, only one flank of the branch reacts to the stimulus of contact, and in fact only that side is sensitive to contact which, when the branch is still very young, and therefore still spirally coiled, forms the convexity. It may here be remarked, however, that there are other plants in which the tendrils are not sensitive merely on one side, but on all sides. To convince ourselves further of the very great sensitiveness of the tendrils of Sicyos or Cyclanthera, it is to be recommended to place gently on the tips of the tendrils, fragments of cotton yarn or small paper riders, a few mg. in weight; curvatures very quickly take place. Other tendrils, *e.g.* those of Vitis, which we shall carefully examine later, are far less sensitive than those we have been considering; loads a few mg. in weight do not, as a rule, cause them to bend inwards at all.

It is of essential importance, and easily proved, that tendrils are not simply sensitive to pressure, impact, or contact, but that only a particular form of contact acts as a stimulus.[2] If tendril-bearing shoots of Sicyos are violently shaken, care being taken to avoid any contact of the tendrils with a solid body, shock curvatures may appear in the tendrils, but the striking effect which is brought about by contact with a solid body is absent. This experiment teaches also that friction against the air does not act on the tendrils as a stimulus. Further, a stream of water directed, *e.g.*, by means of a wash bottle against the irritable side of the tendrils of Sicyos, does not stimulate them. The sensitive leaves of Mimosa pudica, as I satisfied myself, behave quite differently from tendrils, both towards a stream of water and to simple shock [*Erschütterung*]. These leaves are stimulated by any kind of mechanical shock whatever, and if, *e.g.*, we direct a

stream of water against an expanded leaf, the well-known closing movements very rapidly ensue. Tendrils are not irritable to all kinds of mechanical shock; they only react when in their sensitive zone discrete points of limited extent are subjected simultaneously or in sufficiently rapid succession to push or pull. We have therefore to distinguish between impact stimulus [*Stossreiz*], to which, *e.g.*, the leaves of Mimosa react, and contact stimulus [*Contactreiz*], which cause the movements of tendrils.

If a support, *e.g.* a wire or a thin stick, is placed in the neighbourhood of tendrils of Sicyos or Cucurbita, the irritable organs soon come into contact with it owing to the nutation movements already referred to. The contact acts as a stimulus, and the tendrils curve. Owing to this curvature, fresh points of the tendrils come into contact with the support, causing further stimulation, and thus, rapidly or slowly, the tendril comes to wind round the support (see Fig. 166, tendril branch, *d*). When a tendril has grasped a support, notable changes very rapidly appear in the portion of the tendril between the support and the plant. This part of the tendril, viz., as represented in Fig. 166, forms corkscrew-like coils, and for purely mechanical reasons so-called reversal points originate, which separate spirals wound in opposite directions (see Fig. 166, at *W*). I found, *e.g.*, that a Sicyos tendril, which had grasped a support at about 4 p.m. on July 1st, was already coiled corkscrew fashion in the part stretched between the supports and the plant on the morning of the next day; reversal points were also already present. I found further that a Cyclanthera tendril, which had grasped a support, at a high summer temperature already exhibited the first spiral turns between the support and the plant at the end of eight hours. These first turns formed in the immediate neighbourhood of the point of attachment of the tendril to the support. The development of the coils proceeds in the free part of the tendril from the tip towards the base.

Tendrils of our Cucurbitaceæ which have not grasped a support exhibit coiling phenomena like organs which have already attached themselves, but we can readily satisfy ourselves that while the coils form rapidly in attached tendrils, they develop only very slowly in free ones. These facts leave no room for doubt that the accelerated coiling in attached tendrils is due to the contact stimulus to which they have been subjected, and it is moreover clear that propagation of stimulus must play an important part in the

phenomenon, since the spirals are of course formed in the free parts of the tendrils, not in the parts directly touched.

With reference to the mechanics of tendril movements, this much is known with certainty, that when a tendril has been stimulated by contact, and curvatures have been effected, the turgor-expansion of the cells of the now concave side is less than that of the cells of the now convex side. This preliminary difference in turgor-expansion, called into existence by the contact stimulus, then further leads to a difference in the growth of the cells of the concave and convex sides of the tendril. The cells of the latter grow more actively than those of the former, and thus the curvatures induced by the stimulus become fixed. By means of the plasmolytic method (see 59) we are enabled to determine the share taken in the production of the tendril curvatures by turgor-expansion on the one hand and the growth of the cells on the other, and it is instructive to make such experiments. The method is to stimulate the tendrils slightly or strongly, and then, when more or less considerable curvatures have taken place, cut them off and lay them at once in a 20 per cent. solution of common salt. If, under plasmolysis, the curved tendrils completely straighten again, the curvature resulting from the stimulus of contact was only occasioned by a difference in the turgor-expansion in the cells on the concave and convex sides of the tendril. If, on the other hand, complete straightening is not effected by plasmolysis, the participation of growth in the development of the spirals is demonstrated. I have stimulated Sicyos tendrils, and, after they had developed $\frac{1}{4}$, $\frac{3}{4}$, or $1\frac{1}{4}$ turns, subjected them to plasmolysis. The two first tendrils soon straightened completely; the last retained in the salt solution $\frac{1}{4}$ of a turn.[3]

The tendrils of the Cucurbitaceæ are very irritable only at their tips, as was found to be the case with Sicyos tendrils; the irritability gradually falls off as we proceed towards their base. This is undoubtedly related to the fact, of the truth of which I was satisfied from the descriptions of O. Müller,[4] that the base of the tendrils in Cucurbitaceæ is radial in structure, while the nearer we approach the highly sensitive tips of the tendrils the more pronounced becomes their dorsiventrality of structure. We prepare, *e.g.*, a large number of sections from a tendril of Bryonia dioica. At the base the tendril is completely or approximately radial in structure. We perceive the vascular bundles, regularly disposed in the pith, a closed ring of sclerenchyma, the elements

of which, however, need not yet be lignified, then green tissue, which, however, only reaches to the epidermis here and there, since there is an abundance of collenchyma.

If we examine sections from the middle or upper part of the Bryonia tendrils, the dorsiventral structure of the irritable organ stands out more and more clearly. The vascular bundles are crowded together in the ground tissue on the under side of the tendril; the sclerenchyma no longer forms a ring, but an arch on the under side of the tendril. Similarly the collenchyma is here especially abundant, while the green parenchyma constitutes the bulk of the tissue on the upper side of the tendril.

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[1] See Wortmann, *Botan. Zeitung*, 1887, No. 7.

[2] See Pfeffer, *Untersuchungen aus d. bot. Inst. zu Tübingen*, Bd. 1, p. 483.

[3] See H. de Vries, *Arbeiten des botan. Inst. in Würzburg*, Bd. 1, p. 302, and *Landwirthschl. Jahrb.*, Bd. 9, p. 511.

[4] See O. Müller, in Cohn's *Beiträge zur Biologie der Pflanzen*, Bd. 4, p. 120.

191. Experiments with Tendrils of the Ampelideæ.

The branched tendrils of Vitis vinifera are not nearly so sensitive as those of Sicyos and Cyclanthera discussed in 190. These latter, under favourable circumstances, react almost instantaneously and very energetically to the stimulus of contact. Vitis tendrils, even after strong stimulation, curve only slowly. I found on one occasion that a branch of a Vitis tendril which had been drawn several times between the fingers, was distinctly curved, at a high summer temperature, at the end of twenty minutes. In all other cases, especially when the temperature was not so high, the effect of the stimulus was not clearly observable for an hour or two. When a tendril of Vitis has curved owing to transitory stimulations, it slowly straightens again, and is then once more irritable. If we place a thin wooden rod near a Vitis tendril, to serve as a support, the tendril can readily coil round it. The free part of the tendril, between the support and the plant, draws itself together corkscrew-fashion; but this takes place slowly, often not till a few days have elapsed.

The tendrils of Ampelopsis quinquefolia (wild vine) behave in a very characteristic manner. Such a tendril is depicted in Fig. 167; it has not yet made any attachment. The tendril branches are only capable of winding in certain individuals; they are mostly

unable to attach themselves in the manner typical of the tendrils of many Cucurbitaceæ and the tendrils of Vitis. On the other hand, the ends of the tendril branches have the power of producing attachment balls. When, viz., their tips come into contact with the stone or woodwork against which the plant is growing, they at once begin to swell, as a result of the contact stimulus, so

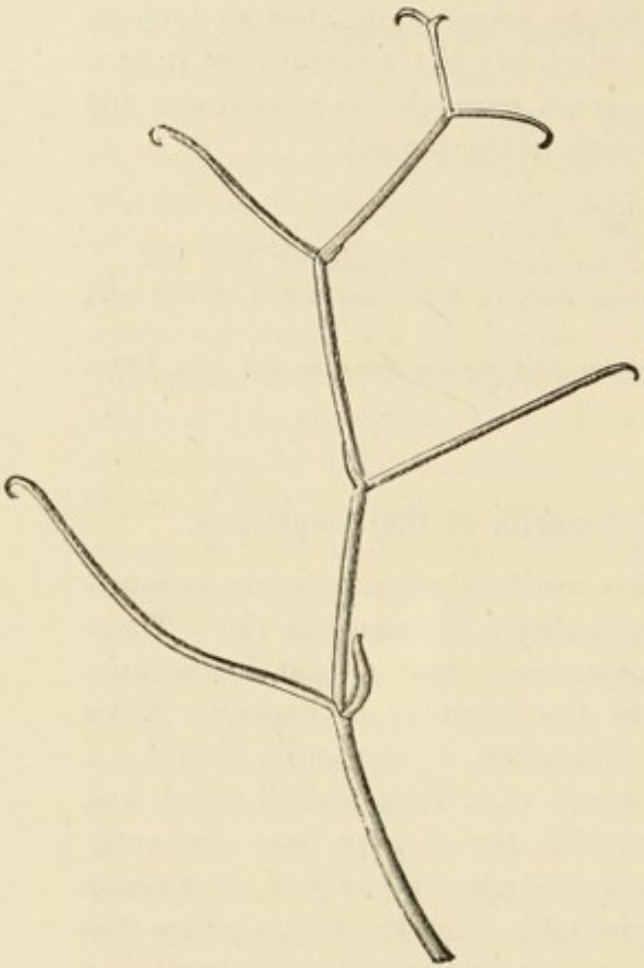

FIG. 167.—Tendril of Ampelopsis quinquefolia unattached.

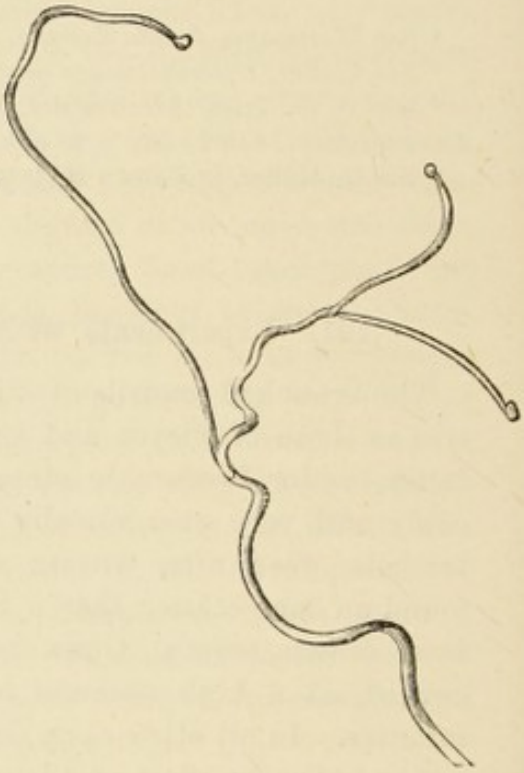

FIG. 168.—Tendril of Ampelopsis quinquefolia, with attachment balls.

as to form the attachment balls. The cells of these last exude a viscid secretion, by means of which the attachment of the tendril tips is effected. In Fig. 168 is drawn a tendril of Ampelopsis, which was removed from a wooden wall after the tips of its branches had just begun to form attachment balls. If an Ampelopsis tendril has in this way fixed itself, there are produced in the part of the tendril between the points of attachment and the plant spiral windings. The development of these, as in tendrils of Sicyos, Vitis, etc., is due to propagation of stimuli, and is inti-

mately connected with the contact stimulus which led to the formation of the attachment balls. If, viz., the Ampelopsis tendrils do not attach themselves, the spiral turns are not produced; the tendrils become withered, even in the course of one or two weeks, and fall off. The contact stimulus, however, leading to formation of attachment balls at the ends of the tendril branches, calls forth not only the formation of the spiral windings referred to, but, owing to transmission of stimuli, it leads to still further changes in the freely extended part of the tendril.

If we examine a thin transverse section of a non-attached tendril branch of Amelopsis, we perceive a large-celled pith, which is surrounded by a vascular bundle circle. The communication between the pith and the green cortex is effected by wide medullary rays, and in the periphery of the cortex, close under the epidermis, is present collenchyma. When the tendrils have attached themselves, their structure undergoes essential changes, simultaneously with the formation of the spiral turns. In the medullary rays interfascicular cambium appears; the wood of the vascular bundles enlarges considerably, till finally a closed ring of wood is produced, whereby the tendrils gain very considerably in firmness and power of resistance, and now become of real service to the plant.[1]

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[1] See Darwin, *The Movements and Habits of Climbing Plants*, and v. Lengerken, *Botan. Zeitung*, 1885, Nos. 22–26.

IV. DORSIVENTRALITY, POLARITY, AND ANISOTROPY IN PLANT ORGANS, AND PHENOMENA OF CORRELATION.

192. Dorsiventrality.

Many plant organs, especially plagiotropic ones (*e.g.* many foliage leaves), are decidedly dorsiventral in construction. Some stem structures are characterised by dorsiventrality, morphological or physiological, and in some of these even the cause of the dorsiventrality is known, and it can hence be induced in them. We will make a few observations in order to gain further information on this interesting subject.

We sow some seeds of Tropæolum majus in summer in good

garden soil contained in flower-pots. We place the pots in front of a window exposed to very bright light. The epicotyls of the young seedlings (we must only grow a few in each pot, so as to avoid their shading each other) at first turn towards the light; they exhibit positive heliotropism. If the plants are allowed to remain undisturbed in front of the window, exposed to the bright light, the positive heliotropism passes over into negative heliotropism. They curve away from the source of light, as also do the new shoots developing later. Their illuminated side (upper side) hence becomes convex. The intense light, therefore, has induced plagiotropism in the Tropæolum shoots, but it is not deep-seated, since the stems of our plants, both with respect to their anatomical structure, and also as regards the position of the leaves arising on them, always present a multilateral or radial character. It is important that the illuminated or upper side of the epicotyl of Tropæolum does not gradually become convex when the objects are exposed to weak light; the negative heliotropism of the structures is not then exhibited; they now curve only in a positively heliotropic manner, towards the light. Moreover, any flank we please of the Tropæolum stem (that, viz., illuminated most strongly) may become the upper side.[1]

A striking case of local induction of dorsiventrality may be proved by experiments with the horizontally growing shoots of Thuja occidentalis.[2] On these shoots four rows of leaves are present: one above and one below (facial leaves); one on each flank (marginal leaves). Shoots of Thuja, grown under normal conditions, are clearly dorsiventral in construction, as we can readily satisfy ourselves by examination of delicate transverse sections. Their mesophyll, *e.g.*, on the upper surface is composed of palisade parenchyma; it consists on the lower side of approximately isodiametric cells (see Frank, Pringsheim's *Jahrbücher*, Bd. 9, Table 16, Fig. 4). If, now, in the early spring, while vegetation is still in a state of dormancy (the experiments which I made began at the commencement of March), horizontal shoots of Thuja, without being removed from the parent plant, are turned upside down and fixed in that position, their morphological lower side being thus directed upwards, it will be found that the shoot ends develop quite normally during the spring, and also, as usual, become dorsiventral in structure. That side of the shoot, however, which, without reversal, would have been morphologically he under side, now becomes the upper, as is clearly indicated,

e.g., by the presence of palisade parenchyma, while the side of the reversed shoots directed towards the soil has assumed the normal character of the under side. The dorsiventrality of Thuja shoots is thus the consequence of local induction. It is brought about by the action of light, not, as Frank sought to show, through the action of gravity.

If, at the time when the buds are bursting, *i.e.* in May, shoots of Taxus baccata, in which the leaves are arranged more or less accurately in two rows, are turned through an angle of 180°, and, without being removed from the parent plant, are tied fast in that position, we observe that the young shoots (not of course the older, already mature ones) after a few days return by torsion to the position which they occupied at the beginning of the experiment. If we rotate the Taxus shoots through 180° before the buds begin to burst (my experiments began in the middle of March), and fix them in this position, then, perhaps chiefly under the influence of gravity, dorsiventrality is soon induced in the developing shoots, which corresponds with the new position, so that the annual shoots after reversal return to the orientation assumed at the time of sprouting. The difference between shoots of Taxus which have developed in the usual manner, and shoots originating from buds whose parent shoots early in the spring were rotated through an angle of 180°, is however essentially this, that in the former the needles diminish in length from the lower to the upper side, while in the latter the longest needles are on the upper side (anisophylly).[3]

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[1] See Sachs, *Arbeiten des botan. Inst. in Würzburg*, Bd. 2, p. 271.

[2] See Frank in Pringsheim's *Jahrbücher*, Bd. 9, p. 147.

[3] See Frank, *Die natürliche wagerechte Richtung von Pflanzentheilen*, 1870, p. 24.

193. Polarity.

Numerous plant structures, especially many shoots, exhibit well-marked polarity. The organisation, or even the physiological behaviour of such structures, points to a distinction between base and apex, and we will first conduct some experiments with a view to obtaining a clear understanding of the noteworthy facts in question (Vöchting).

Water is poured into a glass cylinder to a depth of about 1 cm.

We entirely cover the inside of the cylinder with strips of wet blotting-paper; these must dip into the water at their lower end. The mouth of the cylinder is covered with a sheet of glass. We may now perform our experiments at very different times of the year, and will first see what results we obtain with willow twigs in February or March. If, at this period, we suspend in our cylinder (see Fig. 169) pieces of willow stem (Salix viminalis or Salix fragilis) about 200 mm. in length and 12 mm. in diameter, beset throughout their length with buds as nearly as possible of the same size, the morphological apices of the stems being directed upwards, their basal ends downwards but not dipping into the water, then the buds soon begin to burst, and also the primordia of roots concealed beneath the cortex begin to develop.* In the moist air of the culture cylinder, at a sufficiently high temperature (say 20° C.), and in darkness, the pieces of stem produce in the course of three or four weeks vigorous shoots and roots. We now find, however, that shoots only develop from the buds at the apex, while roots break forth from a very considerable part of the surface. In many experiments, however, which I conducted, I invariably found more or less clearly, my results here harmonising with those of Vöchting,[1] that the roots increase in number and length towards the morphological base of the pieces of stem (see also Fig. 116). If in July we cut out from the middle of a vigorous current year's willow stem pieces say 200 mm. in length, remove their leaves and suspend them in moist air in cylinders, as described, shoots develop at their upper ends. As to the development of roots, that is confined in the young pieces of stem, in contrast with older ones, to their morphological base. We also suspend still older or younger pieces, in March or July, with their morphological apex directed downwards and their morphological base upwards. It again appears that at the apex

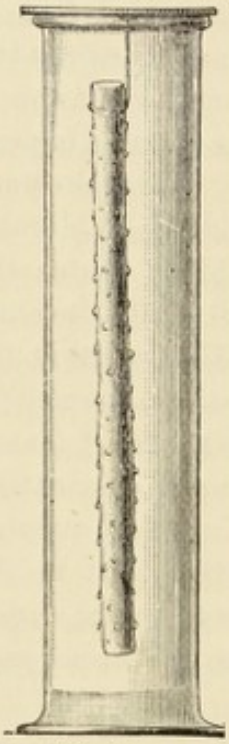

FIG. 169.—Glass cylinder in which is suspended a sprouting piece of a willow twig.

* It must be emphasized that the root primordia are uniformly distributed under the cortex throughout the entire twig.

shoots are produced, while at the morphological base especially numerous and vigorous roots are formed. The experiments with inverted pieces of stem thus teach that the action of gravity cannot be the immediate cause of the phenomena we have observed in connection with the development of shoots and roots.

It is certain that the polarity of plant structures, *i.e.* the differentiation into base and apex, is not due to mysterious vital forces, but that it owes its origin to external forces. We have every reason to suppose that gravity was, in the first instance, the chief cause of that polarity, and we may form for ourselves an idea somewhat as follows of the problem before us. When gravity always acts in the same direction and for numberless generations on plants, an inheritable peculiarity may finally result, owing to summation of effects, which, in fact, we designate as polarity. Polarity would accordingly be regarded as an after-effect phenomenon, induced by gravitation and stretching beyond the life of the individual, as a phenomenon of inherent or stable induction, or, as we are accustomed to say, as an inheritable disposition. From this point of view it would also be intelligible that gravity is unable to exert any essential direct influence on the development of buds and roots in structures having well-marked polarity.* But gravity does nevertheless exert, under particular circumstances, a striking influence on them, as we may easily satisfy ourselves by an experiment, which I conducted as follows. A fairly large zinc box was half filled with water. Over the surface of the water were then arranged horizontally a number of pieces of willow stem, 200 mm. in length and 12 mm. in diameter, as can easily be effected by supporting them at their ends on suitable supports projecting out of the water. The zinc box I closed not completely air-tight with a cover, so that the research material was in darkness. The experiments, which were made in March and April, showed that the buds developed preponderatingly at the morphological apex of the pieces of stem, while the roots formed chiefly at the base. It is, however, of special significance for us here that the former developed chiefly on the upper, the latter on the lower side of the horizontally-lying stems, a result without any doubt dependent on the action of gravity. In investigations of this kind, a number of pieces of

* All shoots do not exhibit such decided polarity as willow twigs, and such are then accessible also to local induction. See Sachs, *Lectures on Plant Physiology*.

stem must always be employed side by side, because different specimens behave very differently.

It may further be remarked incidentally that, as Vöchting has specially shown, light exerts an important influence on the development of roots in willow twigs. If we suspend pieces of willow stems, as described, in glass cylinders, cover one cylinder with a black receiver, leaving the other exposed to diffuse daylight, we find that in the light fewer roots break from the cortex than in the dark, and that the root development is also completed more slowly in the light than in the dark.

In many plant structures polarity as well marked as that of willow stems is to be observed. I have, *e.g.*, placed potato tubers in a box, in winter, so that they were excluded from the light.[2] The apices of several tubers were directed upwards, those of others downwards, but always vigorous shoots developed in abundance only at the morphological apex, *i.e.* at the end of the potato which is opposed to the former point of attachment to the parent. Under the conditions described the shoots can only obtain the water necessary for their formation, as also the necessary food stuffs, from the tuber (see Fig. 170).

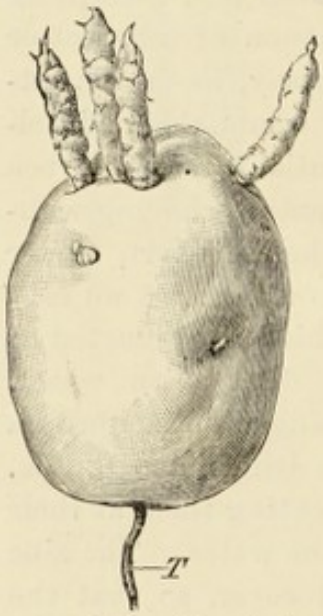

FIG. 170. — Germinating potato tuber. *T*, runner.

If we suspend in glass cylinders containing some water pieces of root, *e.g.* of Ulmus campestris, 100–150 mm. long, and 10 mm. in thickness, with their morphological apices directed upwards or downwards, and then exclude the light, they always produce adventitious shoots at their morphological base only. New roots are more rarely produced at the morphological apex; they do not originate here at all readily. Shoots, as we have seen, form at their morphological apex new shoots; roots, on the other hand, yield shoots at their morphological base.

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[1] See Vöchting, *Ueber Organbildung im Pflanzenreich*, 1878. See further Vöchting, *Ueber Transplantationen am Pflanzenkörper*, Tübingen, 1892.

[2] See Detmer, *Sitzungsber. d. Jenaischen Gesellsch. f. Naturwissensch. und Medicin*, 1884, p. 5.

194. Anisotropy.

The direction which plant structures take in growth and finally maintain, by no means depends on accidental circumstances, but in a definite way stands in a causal relation to a series of internal and external growth determinants. Hence it is even in many cases possible to set forth, at least to a certain extent, the causes which lead a plant structure under given conditions to take up one and no other direction of growth, and a number of these cases may here find special mention.

If we cultivate seedlings of Phaseolus in the manner described in 172 in a zinc box, behind a sheet of glass, it is easy to make out that the lateral roots of the first order make a definite angle with the main root, which we term the geotropic limiting angle. Our culture has been placed in the dark, and when a number of lateral roots have developed we indicate the direction of their apices by making ink-lines on the outside of the sheet of glass. We now expose our apparatus to diffuse daylight, keeping the temperature constant. The tips of the lateral roots undergo a considerable change in their direction of growth, as can be unmistakably seen even at the end of twenty-four hours. Even in the light of course the roots do not grow vertically downwards, but still the geotropic limiting angle of the newly formed parts of the roots is considerably less than that of the parts which originated in darkness. Light, therefore, is able to influence in a definite way the geotropic behaviour of roots.

If in spring we examine the soil in the neighbourhood of flowering plants of Adoxa Moschatellina, we find that it is traversed by numerous ivory-white rhizomes of the plant. They run horizontally in the soil, and the question arises, what conditions bring about the plagiotropism of the organs. We cut off the ends of some of the rhizome branches to a length of several centimetres, stick them with their lower ends in moist soil contained in a flower-pot so that their apices are directed upwards, and put them in a dark place under a bell-glass. After a few days (in my experiments after two or three days) the growing parts of the rhizome tips are directed horizontally, and the growth curvature, as follows from our experiment and others, is due exclusively to the action of gravity. Stress must, however, be laid on the fact that the geotropism of the rhizome of Adoxa does not cause it to assume a vertical but a horizontal direction.[1]

When the pieces of Adoxa rhizome, placed with their basal ends in soil, are unilaterally illuminated, from above or better from the side, their growing apices in the course of a few days curve vertically downwards. The downward curvature takes place sometimes towards one side, sometimes towards the other. It shows on definite relations to the incidence of the light. The light here also influences the geotropic behaviour of the organs in a characteristic manner (heterogeneous induction of Noll).

It is further instructive to make the following experiment, which I carried out with shoots of Sida Napæa deprived of their leaves, since it teaches that temporarily even geotropic after-effects may be of importance in determining the direction of growth of plant structures (see also 173). We stick the lower end of a shoot of Sida into moist sand heaped against one wall of a zinc box inside, and leave the shoot in a horizontal position for $1\frac{1}{2}$ hours at a temperature of about 20° C., until a geotropic upward curvature has just begun. We now rotate the shoot 90° to the right or left. At the end of two or three hours from this time, we find that the curvature of the shoot in the horizontal direction has, owing to geotropic after-effect, become very considerable. With this curvature, however, has been combined another, an upward one, induced by the direct action of gravity, so that the shoot curves obliquely upwards.

It is of considerable interest to prove that, in some plant structures, the direction of growth is dependent, owing to their heliotropic peculiarities, on the position of the sun. If we examine flowering specimens of Tragopogon orientalis growing in the open, we shall find that the flower heads are only open in the morning. They close in the course of the forenoon. In the hours of the morning, the flower heads are directed towards the east; during the day they follow the course of the sun towards the south and on to the west, as I often observed, and at night direct themselves straight upwards. The movement of the heads of Tragopogon is effected by the stem structure supporting them. At the time of blooming this is in a state of intense heliotropic irritability, and always grows more rapidly on the side which for the time being is shaded than on that turned directly towards the sun, so that the movements described must of necessity result.[2]

Radially constructed organs are very generally orthotropic; they grow straight upwards or downwards. Plagiotropic organs, on the other hand, are usually dorsiventral.

We cover the bottom of a large zinc box with moist sand, heap up part of it to form a wall against one of the sides of the box, into which we can stick the lower end of the object under investigation, so that the rest of it does not touch the sand and is directed horizontally. We experiment with structures which under normal conditions clearly exhibit plagiotropism, and first select for examination young stems of Pyrus Malus, or runners of Potentilla reptans or Ajuga reptans. Several specimens must always be taken, as like one another as possible, and about 15–20 cm. long. After their leaves have been removed, they are placed in the zinc box, some with their upper side uppermost, others with their under side uppermost. We now place a cover on the box, and leave the stems in the dark space for a good time (*e.g.* twenty-four hours). We then find that all of them have curved upwards, those however placed with their lower side uppermost more strongly than the rest, as may be more exactly determined by ascertaining the radius of curvature. This upward curvature is in each case the result of negative geotropism, but when the stems are in the normal position it acts in opposition to epinasty, while the stronger curvature in the reversed stems must be regarded as the resultant effect of geotropism and epinasty acting simultaneously in the same direction. Epinasty, *i.e.* the more vigorous growth of the upper side of plant structures, is to be referred, according to H. de Vries, to internal growth determinants, a view to which we shall return.

The flower-bearing shoots of Atropa Belladonna, directed under normal conditions horizontally, are remarkably epinastic. If we cut off such shoots, place them in water, and leave them in the dark for say twelve hours, we shall find that the shoot ends, at the commencement of the experiment vertical, have directed themselves horizontally (see Fig. 171).

I have also examined young ends of plagiotropic shoots of Corylus Avellana, sticking them in the manner already described into moist sand contained in a zinc box. In these experiments, frequently repeated in the course of the summer, I always found, what is not in harmony with the results of H. de Vries, that shoots deprived of leaves, and placed upper side upwards, curved downwards, while shoots fixed lower side upwards curved strongly upwards. In this case, therefore, we have strong epinasty and weaker geotropism co-operating.

According to my experiments, shoots of Corylus humilis placed

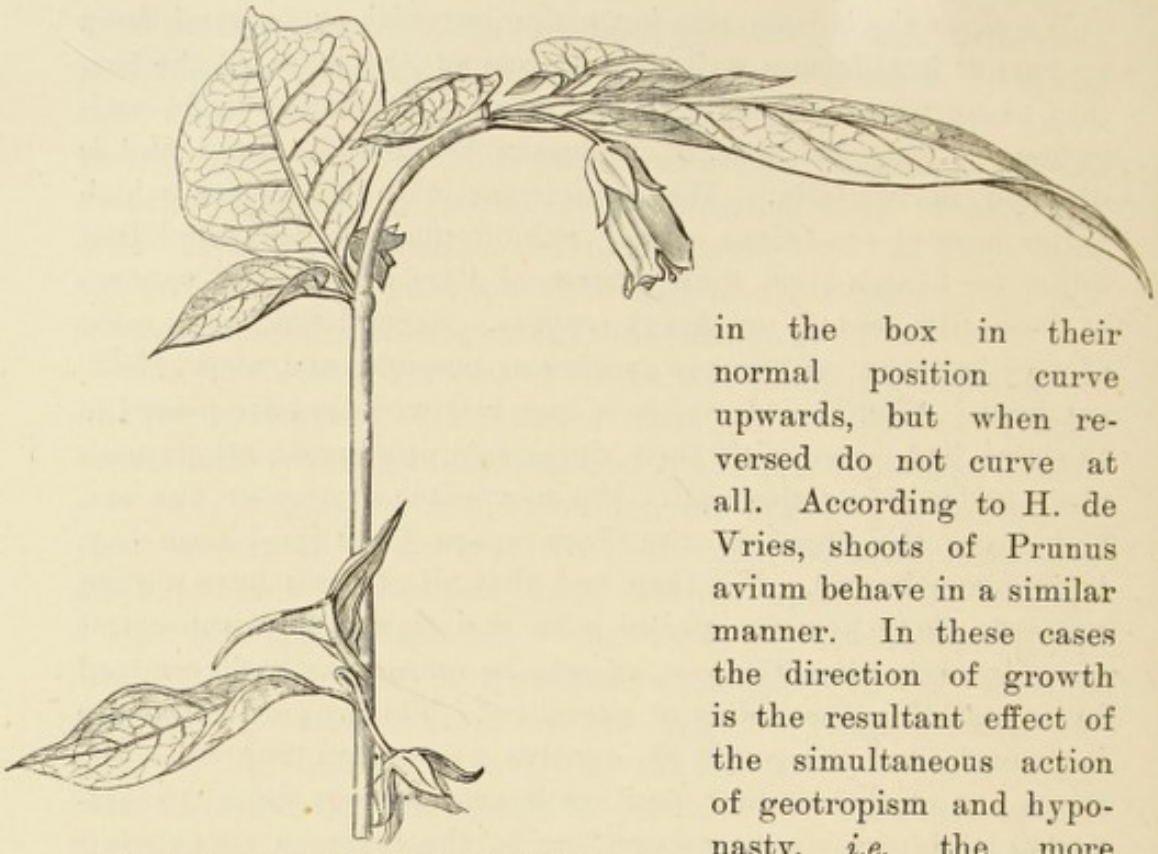


FIG. 171.—Shoot of Atropa Belladonna, the terminal portion of which has curved epinastically.

in the box in their normal position curve upwards, but when reversed do not curve at all. According to H. de Vries, shoots of Prunus avium behave in a similar manner. In these cases the direction of growth is the resultant effect of the simultaneous action of geotropism and hyponasty, *i.e.* the more active growth owing to internal causes of the under side of the shoot.

We will now make experiments with leaf-stalks and midribs of leaves similar to those we have conducted with shoots. If, *e.g.*, we experiment with growing leaf-stalks of Calla palustris and Petasites, with the main leaf-stalks of the pinnate leaves of Sambucus nigra or Juglans regia (always without the lamina), or with midribs of Sambucus leaves, and stick them with their ends in the sand wall of the zinc box, in the normal position or reversed, we shall always find that they are negatively geotropic and more or less strongly epinastic. I tested especially the behaviour of Calla, and of the midribs of Sambucus leaves. In the normal position they curved downwards, in the reversed position strongly upwards. If leaf-stalks or ribs are placed horizontally in the zinc box, on their side, their median plane therefore being disposed horizontally, then negative geotropism must strive to bring about an upward curvature in a vertical plane, while epinasty tends to produce a curvature in a horizontal plane. The resultant curvature actually taking place, must therefore be in an oblique plane.

If growing leaf-stalks, some of them deprived of their laminæ the rest not, are placed in a horizontal position in the zinc box, care being taken that the upper side of the organs is always upwards, then the stalks provided with laminæ, if their epinasty is not too great, do not curve at all, or, at any rate, curve much more feebly upwards than those freed from their laminæ. Here, then, circumstances of loading have influenced the result of the experiment. Many further particulars respecting these phenomena, and also with reference to the determination of the amounts of growth taking place in connection with the curvatures of shoots and leaves, will be found in a valuable memoir of H. de Vries, which deals with the subject.[3]

It has already been mentioned that leaf-blades, leaf-stalks, and many shoots, have the power of growing more energetically on their morphologically upper surface than on their lower surface. This epinastic behaviour of these organs is of great importance in determining their normal plagiotropic orientation, and H. de Vries advocates the view that the epinasty is the result of internal growth determinants. I have been led to other results by investigations as to the causes of epinasty in the leaves of Phaseolus multiflorus and Cucurbita.[4] We grow seedlings of Phaseolus and Cucurbita in flower-pots in complete darkness. At a temperature of about 20° C., the hypocotyl of Cucurbita develops to a considerable length in about ten days; the cotyledons are directed straight upwards, their upper surfaces being in close approximation. In about fourteen days the epicotyl of Phaseolus has grown to a considerable length, and the long-stalked primordial leaves present a shell-like appearance owing to hyponastic growth, *i.e.* stronger growth of their lower than their upper surface. If the seedlings are now exposed to bright diffuse daylight, the primordial leaves of the Phaseolus seedlings lose their shell-like form, owing to epinastic growth. Similarly, in the cotyledons of Cucurbita, the upper side now grows more actively, so that they pass from their original orthotropic position into a plagiotropic one. But it needs the light to call forth the energetic growth of the upper side of the leaf; in the dark it does not take place. The epinasty is not a spontaneous but a paratonic nutation phenomenon, and in future we shall not briefly speak of epinasty, but of photoepinasty of the leaves. If, after keeping them in the dark till they have reached the stage of development above described, we bring our Phaseolus or Cucurbita seedlings into diffuse light

for three to five hours, no photoepinastic movement will yet have taken place. If, however, we now put them back into the dark, the leaves spread out in the course of six to twelve hours. Here, then, we have an example of photoepinastic after-effect.

The seedlings, grown in the dark and not too old, always exhibit photoepinastic nutations when illuminated, whether the light strikes them from above or in some other direction. The leaves strive to place themselves approximately at right angles to the incident rays of light, and in experiments with Cucurbita it is easy also to prove the fact that here—and the same thing obtains in other cases also—one organ (viz. the hypocotyl) exerts a material influence on the final orientation towards the light of other organs (the cotyledons). The intense positive heliotropism of the hypocotyl plays an important part in determining the light position of the leaves, when the seedlings are illuminated only from one side.

If growing leaves are brought out of their normal position as regards the light into an abnormal one, they endeavour to return to their former position. I have made a series of experiments to prove this, and it will be instructive to repeat them. We cultivate a few plants of Cucurbita in flower-pots. The experiments begin when the plants have formed several leaves. Some of the leaves are raised so that the petiole is vertical, and the tip of the lamina points upwards. The leaves are bound to small sticks, the thread being brought close below the lamina at the end of the petiole. If we arrange the plants so that with unilateral illumination the under sides of the leaves are directed towards the light, then the leaf-blades, owing to photoepinastic movements, will speedily assume a normal inclination to the light. If, with unilateral illumination, we arrange a flower-pot, in which plants of Cucurbita are growing, in such a way that the free leaf-stalks, as also the laminæ with their under sides directed towards the light, are vertical, then heliotropic and photoepinastic movements take place, and the organs return to the normal light position. If we take into the dark plants of Cucurbita which have developed under normal conditions, taking care that some of their leaves (stalk and blade) are vertical, the blades perform photoepinastic after-effect movements until they reach a horizontal position.

It is of special interest in connection with questions relating to the natural orientation of plant structures, and their anisotropy, to make a few observations and experiments on the growth of the

shoots of Hedera Helix. The shoots of the ivy are markedly dorsiventral and plagiotropic, with the exception of the flower shoots, which are not developed until the plants have attained a considerable age, and which are orthotropic. If free-swinging ivy shoots, about 30 cm. in length, are cut off and planted in flower-pots, and after six to eight weeks, when they are well rooted, are bound to a vertical stick, we can, under suitable conditions, observe interesting phenomena at the summit of the shoot projecting beyond the end of the rod.

In August I placed Ivy plants, obtained as above, in front of a window with a north aspect, and found that the shoot ends speedily turned away from the window. They grew horizontally inwards into the room, and after four weeks presented the appearance depicted in Fig. 172. The shoots performed negatively heliotropic and photoepinastic curvatures (as to the *modus operandis* however, further investigations are necessary). The illuminated side of the shoot was consequently forced to become convex and bring the shoot ends into a horizontal position. This having been effected, they did not curve still further downwards, since now negative geotropism (the geotropic properties of ivy shoots have been specially investigated by Sachs) was able to assert itself very energetically. It appears, therefore, that the horizontal position of ivy shoots is the resultant of the directive influence of light on the one hand, and gravity on the other (?). The same factors also determine the direction assumed by the shoots of ivy plants growing in nature.

In the preceding pages we merely discussed in the first place certain properties of leaf structures and many shoots. We have now sought to explain the normal position of leaves and shoots by reference to these properties. We conceived the normal position of the organs as brought about by the co-operation of various forces. Thus the usual orientation of leaf-blades, *e.g.*, will be essentially due to the co-operation of their photoepinasty on the one hand and their negative geotropism on the other.

It is, in fact, certain that the normal position of leaf-stalks, and also certainly of many shoots, can be explained in the manner indicated. But it is precisely as regards the leaf-blades that the method is unsatisfactory. Vöchting, Schwendener, and others have shown that neither geotropic movements of the laminæ, nor conditions of loading, need directly and of necessity be concerned in bringing about their fixed light position. On the contrary,

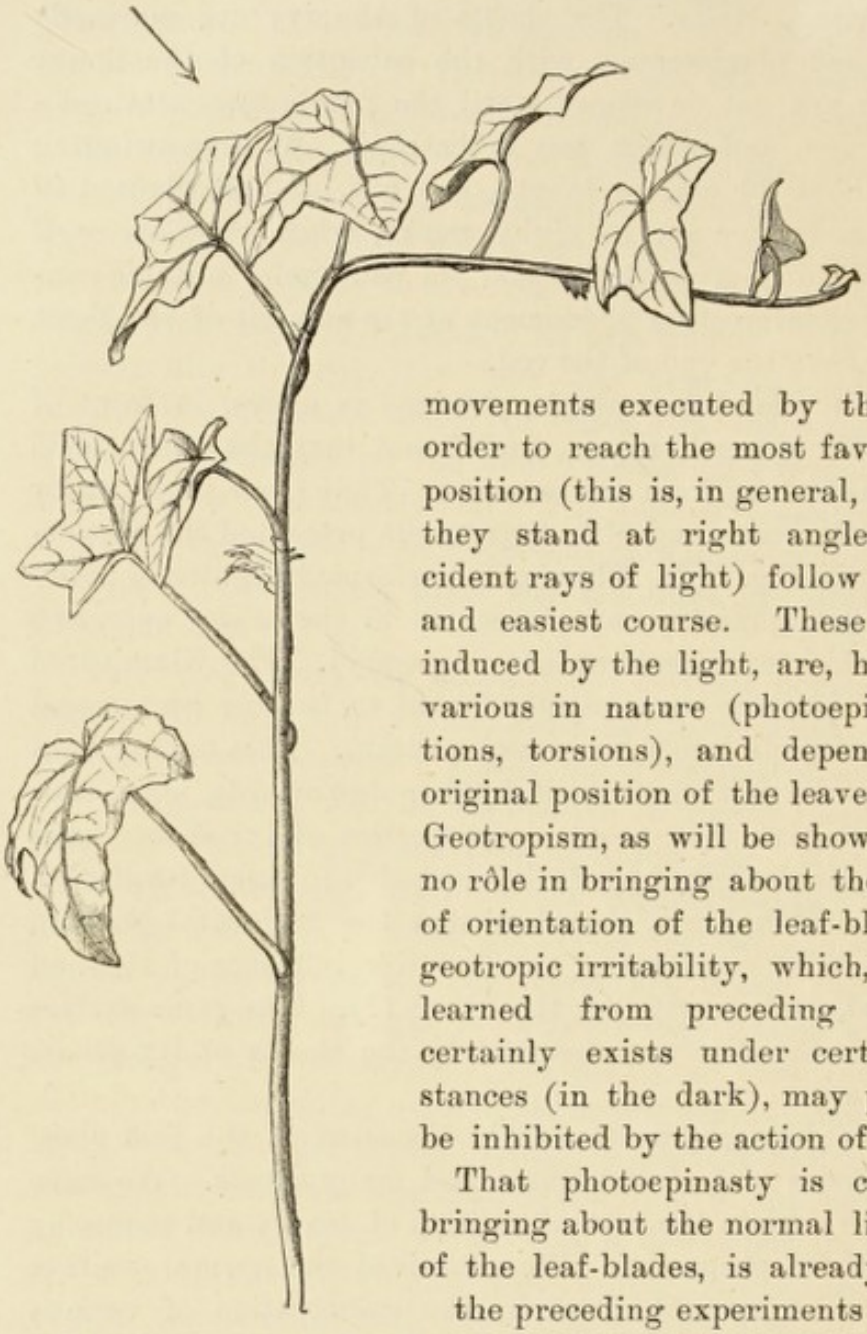


FIG. 172.—Shoot of Hedera Helix subjected to unilateral illumination. Its terminal portion has curved away from the source of light.

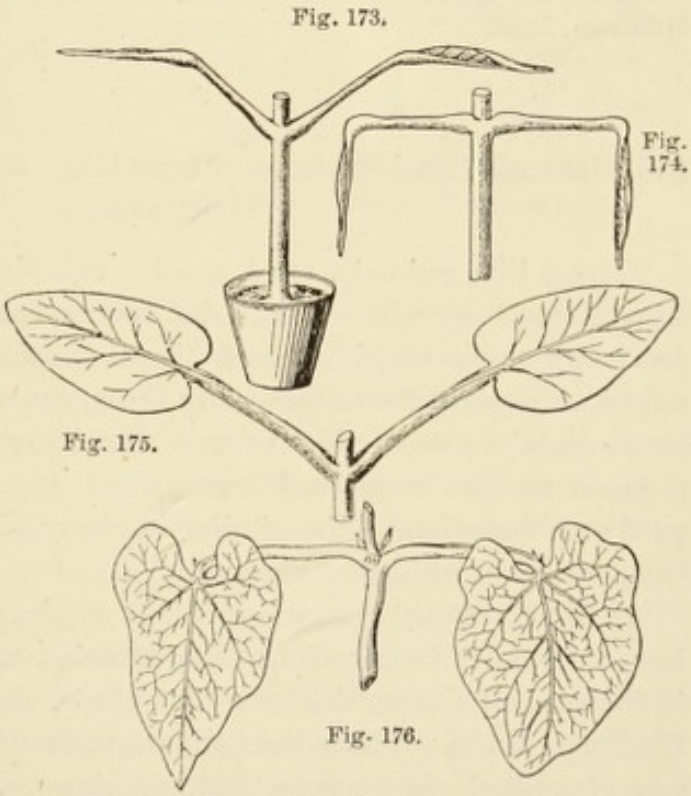
the light must in this case be regarded as the determining factor, as I also now admit.[5] The movements executed by the laminæ in order to reach the most favourable light position (this is, in general, one in which they stand at right angles to the incident rays of light) follow the shortest and easiest course. These movements, induced by the light, are, however, very various in nature (photoepinastic nutations, torsions), and dependent on the original position of the leaves themselves. Geotropism, as will be shown, need play no rôle in bringing about the movements of orientation of the leaf-blades. Their geotropic irritability, which, as we have learned from preceding experiments, certainly exists under certain circumstances (in the dark), may perhaps even be inhibited by the action of light.

That photoepinasty is concerned in bringing about the normal light position of the leaf-blades, is already clear from the preceding experiments with Phaseolus and Cucurbita plants. It causes, *e.g.*, the unfolding, on access of light, of leaves which in many plants are in the dark coiled shell-fashion.

Seedlings of Cucurbita which have been grown in the dark till their hypocotyl has attained a fair length, and the cotyledons are erect, are placed in a box fairly high at the back but low in front, so that the cover, which is formed by a sheet of glass, may be inclined at an angle of about 45°. The box is papered inside with dull black paper. To prevent heliotropic nutations, the hypo-

cotyls are bound to rods standing in the soil of the flower-pots. The seedlings are now arranged so as to present the broad faces of the cotyledons towards the window in front of which the box is placed. After a time it will be found that both cotyledons have placed themselves perpendicular to the incident rays of light. The front one must in so doing have described an arc of 135° downwards, the back one an arc of 45° backwards. If we wished to explain these movements as due to the action of gravity (and other forces) we should have to make the at least unwarrantable assumption that the geotropic irritability of the two cotyledons of a plant is different. The following experiments, however, serve well to prove that the light position of the leaf-blades can be brought about solely by the action of light.

We grow seedlings of Phaseolus in flower-pots. They are ready for use when the primordial leaves have unfolded, but are still in very vigorous growth. If we illuminate the plants from above, then the leaves take the position indicated in Fig. 173. If now we make the light fall not vertically from above, but horizontally, and nearly vertical to the plane of insertion of the leaves, a torsion of about 90° is effected by the pulvini of leaf-stalk and blade, and the blades then stand once more at right angles to the incident rays of light (see Fig. 175). Now plants with the leaves in the position represented in 173 are slowly rotated in such a way that their shoot axes move in a vertical plane, while the light falls fairly vertically to the plane of insertion of the leaves. The effect of gravity is eliminated, and the epinasty of the pulvini of the leaf-stalk



FIGS. 173–176.—Young Phaseolus plants. (After Schwendener.)

and blade, thus left unhampered, at first causes the leaves to assume the position indicated in Fig. 174. After a short time, however, in the pulvinus of the blade, and possibly also in the leaf-stalk, there appears a curvature (not a torsion) whereby the lamina is directed obliquely frontwards to the light (see Fig. 176). We see, therefore, that the leaves, even in complete absence of gravitational influence, can come to a fixed light position. And in doing so, as is specially to be emphasised, no torsions are effected. Under other circumstances, however, as we have seen, torsions play an important rôle in connection with the movements of orientation of leaves.

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[1] See Stahl, *Berichte d. Deutschen botan. Gesellsch.*, Bd. 2, H. 8.

[2] See Wiesner, *Denksch. d. Akad. d. Wiss. in Wien*, Bd. 43.

[3] See H. de Vries, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 1, p. 223.

[4] See Detmer, *Botan. Zeitung*, 1882, No. 46.

[5] Literature: Frank, *Die natürliche wagerechte Stellung der Pflanzentheile*, Leipzig, 1870; C. Darwin, *The Power of Movement in Plants*; F. Darwin, *J. Linn. Soc.*, Botany, Vol. 18 (Dec., 1880); Schmidt, *Dissertation*, Berlin, 1883; Noll, *Arbeiten d. botan. Inst. in Würzburg*, Bd. 3; Krabbe, Pringsheim's *Jahrb.*, Bd. 20; Vöchting, *Botan. Zeitung*, 1888; Noll, *Ueber heterogene Induction*, Leipzig, 1892; Schwendener and Krabbe, *Abhandl. d. Königl. preuss. Akad. d. Wiss.*, Berlin, 1892; Noll, *Die Orientirungsbewegungen dorsiventraler Organe*, München, 1892.

195. General Considerations Respecting the Rigidity of Plant Structures.

Plants, like animals, need special contrivances to enable them to withstand certain external influences and maintain their form. In many cases turgidity and tissue tensions play an important part as a means of strengthening the organism, but Schwendener's researches[1] have further proved that plants are characterised by a more or less connected mechanical system (stereome), which performs functions quite similar in many respects to those of the bony skeleton of higher animals.

As the ultimate organs (stereides) of the mechanical system, we have especially to take into consideration the sclerenchyma fibres in the ground tissue, the true bast fibres, the collenchyma, and the libriform fibres. The sclerenchyma cells and the true bast fibres are elongated elements, whose membranes, frequently lignified, are much thickened (see Fig. 177). Like these in many respects

are the libriform fibres of the wood, whose strongly thickened walls are frequently provided with slit-like pits. The secondary wood of Tilia, for example, is very rich in libriform fibres. Finally, in many young structures, still in a state of growth, the collenchyma is to be regarded as mechanical tissue. It is easily recognised by the characteristic thickening of its membranes, which is for the most part confined to the angles of the cells (see Fig. 178).

The mere presence of stereides, however, is by no means sufficient to ensure the rigidity of organs with reference to flexion, tension, and pressure; the stereides must at the same time have a very definite arrangement in the rest of the tissue.

To establish constructions resistant all round to flexion (especi-

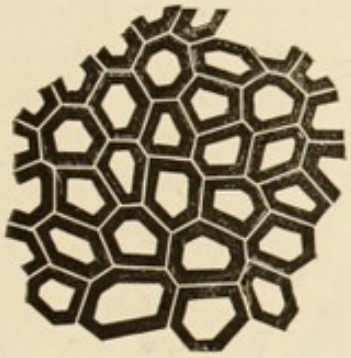

FIG. 177.—Sclerenchyma in cross section.

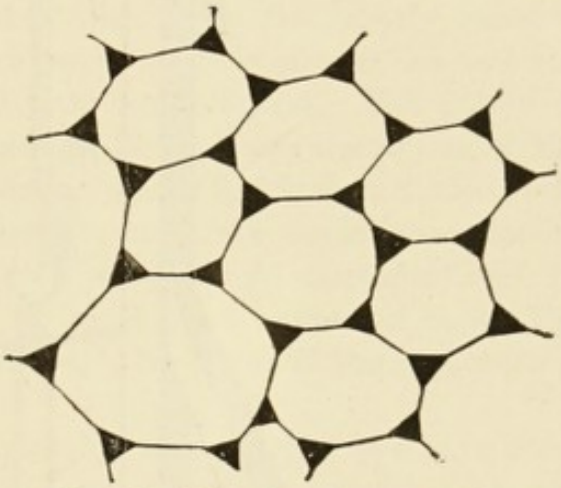

FIG. 178.—Collenchyma in cross section.

ally stems), it is sufficient if the mechanical elements are arranged in a circle at the periphery of the cross section. The packing between the bands is composed of parenchyma and other tissue. In many organs, especially in leaves, which do not need to be made resistant on all sides to flexion, the mechanical tissue is only distributed on the upper and lower sides. Constructions resistant to tension are secured by having the mechanical elements arranged in the organs not peripherally but, on the contrary, centrally, and in a single compact mass (roots, rhizomes). For constructions resistant to pressure it is not a matter of indifference whether they are exposed to longitudinal or radial pressure. Roots and rhizomes developing in the soil are exposed to a radial pressure, in addition to a considerable tension; they are hence

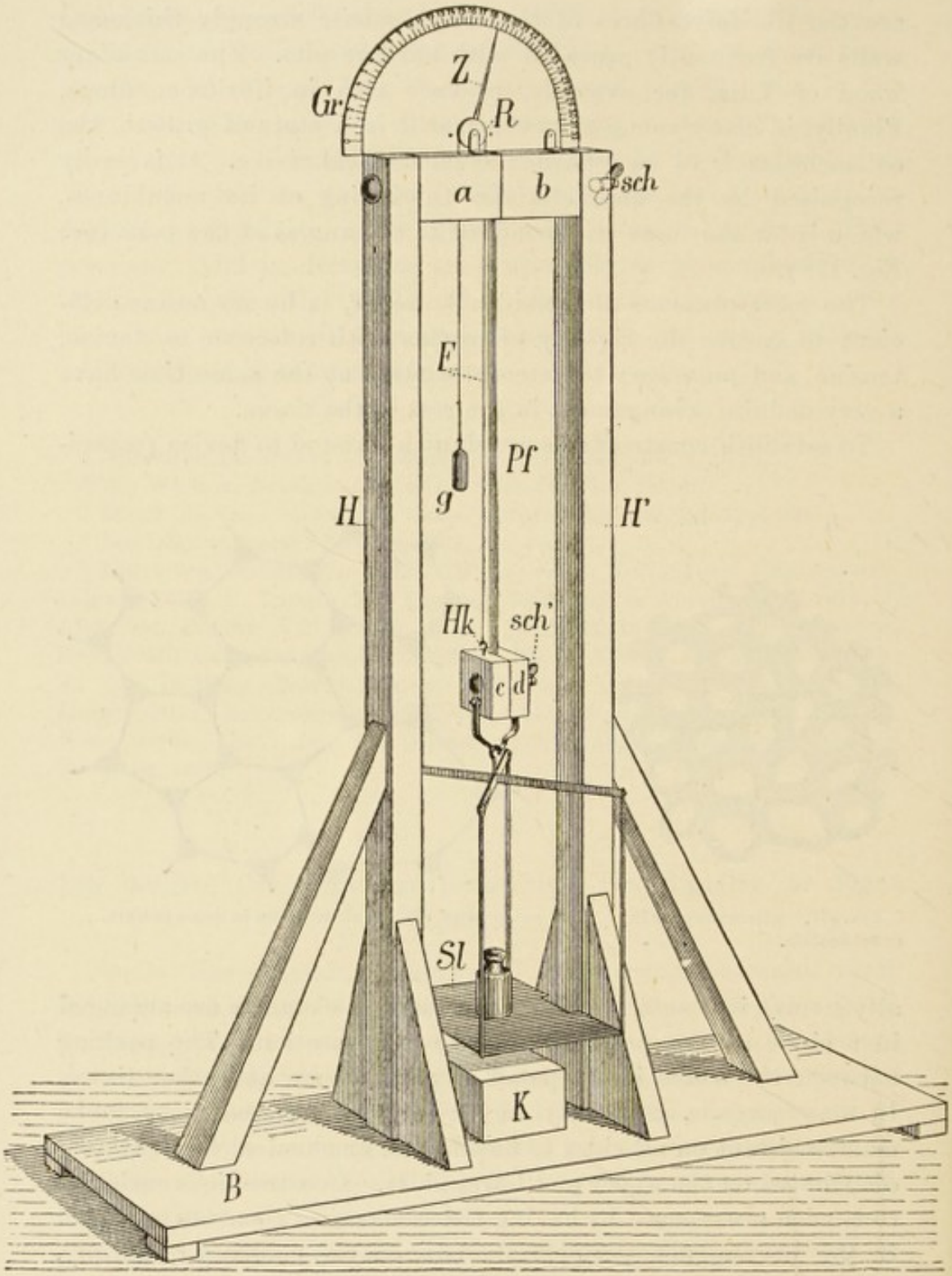

FIG. 179.—Apparatus for determining the extensibility, elasticity, and rigidity of plant tissues.

frequently provided with a peripheral mantle of mechanical tissue, besides other stereome.

In order to discuss properly the efficiency of the mechanical

tissues in the plant organism, it is of importance to make ourselves somewhat accurately acquainted with the rigidity, tenacity, and elasticity of the stereome; this can be done by means of the apparatus depicted in Fig. 179, which I have recently had made. It is throughout very solid in construction. It consists of a board *B*, on which are fixed the two wooden uprights *H* and *H'*, about 86 cm. in height, and 14 cm. apart. The structure to be investigated, about 300–400 mm. long, is clamped above between the blocks of wood *a*, *b*, and below between the blocks *c*, *d*, by tightening the thumb-screws *Sch* and *Sch'*. The scale pan *Sl*, suspended from *c* and *d*, takes the weight employed to stretch or rupture the object under examination. To the hook *Hk* on the block *c* is attached a silk thread *F*, which is taken through a perforation running between the blocks *a* and *b*, and bisected by their surface of contact, over the lightly running pulley *R*, and back through a perforation in *a*. At its end the thread *F* carries the weight *g* to keep it taut. The pulley *R* carries the pointer *Z*, whose length is ten times the radius of the pulley. The pointer moves over a scale *Gr*, which is divided into millimetres. The extension of the structure under examination is therefore read off on the scale with tenfold magnification. In using the apparatus, blocks of wood must be placed below the scale pan, so that the fall will only be slight if the structure is ruptured.

We may first suitably employ for examination, as I have done, a strip say 400 mm. in length and 2 mm. in breadth from the middle part of the leaf of Phormium tenax. Also long internodes from rye haulms are suitable. We do not, however, put entire internodes into the apparatus, but strips obtained by splitting them longitudinally into four parts.

Such a strip having been put into the apparatus, we stretch with a load of 1 kg., determine the elongation produced, and remove the weight again. If the structure now recovers its original length, its limit of elasticity has not been overstepped. We now repeat the experiment with a load of 2·3–10 kg., till finally, at a certain load, the strip gives way. If we are experimenting with strips of tissue, which, like those from the leaf of Phormium, have bast fibres or sclerenchyma as mechanical tissue, we shall find that, even with a large load, they remain almost completely elastic, while collenchymatous structures, although they may have a considerable amount of rigidity, are only very incompletely

elastic, and therefore remain permanently elongated after being stretched. To define accurately the rigidity of a strip of tissue, we must express it in terms of 1 sq. mm. of stereome area. If, *e.g.*, the mechanical tissue in a transverse section of a leaf of Phormium, when magnified thirty times,* occupies a surface of 900 sq. mm., the actual area of the stereome in the transverse section is 1 sq. mm. If the strip of Phormium ruptures at a load of 15 kg., then the rigidity of 1 sq. mm. of Phormium stereome would be expressed by 15. Frequently, however, there are very considerable difficulties in determining the real area of the stereome in transverse section, even approximately. In many cases we have to be content with rough estimates.[2]

Our object is best attained by sketching thin sections from the weakest part of the object, *e.g.* from the place where the rupture took place on loading, under slight magnification (say thirty-fold), and then measuring the area of the mechanical elements on the sketch by means of the planimeter (to be obtained, together with directions for use, from J Kern, Aarau, Switzerland, for 45 mk.), or by means of millimetre paper. If necessary, the relation of lumen to wall-thickening may be taken into consideration. In making the measurements solid xylem strands are to be included in the result.

In many cases, particularly in testing the rigidity of collenchyma, it is advisable to observe the following method of procedure for determining area in transverse section of the mechanical tissue. We draw exactly, under high magnification, a large number of collenchyma cells, using for the purpose good writing paper, which we may assume to be approximately of the same thickness at all points. We now determine the weight of the paper covered by the drawing, cut out the cell lumina by means of a knife, and weigh the network of paper left. Comparison of the two weights gives the ratio of the total area to that of the cell-walls. If we now sketch under slight magnification the outlines of the collenchyma strands of a transverse section of the object, and determine the corresponding area, we can at once estimate the total area of the wall substance of the mechanical tissue. Good material for investigation of the rigidity of collenchyma is

* To determine exactly the magnification employed, we sketch on the paper by means of the camera lucida the lines of an objective micrometer divided into 0·01 mm. We now measure the distance between the lines by means of a millimetre scale, and can then at once deduce the magnification.

afforded, *e.g.*, by strips from the stems of Levisticum officinale and Fœniculum officinale.

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[1] See Schwendener, *Das mechanische Princip im anatomischen Bau der Monocotylen*, Leipzig, 1874.

[2] Further literature: Ambronn in Pringsheim's *Jahrbücher*, Bd. 12; Haberlandt, *Physiol. Pflanzenanatomie*, Leipzig, 1884, p. 96; Tschirch in Pringsheim's *Jahrbücher*, Bd. 16; Lukas, *Sitzungsber. d. Akad. d. Wiss. zu Wien*, Bd. 85; Sonntag, *Landwirthschl. Jahrb.*, Bd. 21.

196. The Arrangement of the Mechanical Tissues in Structures Resistant to Flexion Tension and Pressure.

We shall here consider a series of objects, the majority of which I have myself investigated, and which serve well for studying the arrangement of the stereome. We only need to submit transverse sections of the structures to microscopic observation, and we begin with organs constructed to resist flexion.

In the leaf stalk of Begonia the mechanical tissue consists of a well-developed ring of collenchyma lying immediately below the epidermis. This encloses parenchyma, in which are distributed the vascular bundles. In the stem of Lamium album, bands of collenchyma run in the four angles of the stem, forming two pillars combined crosswise. The transverse section of the stem of Falcaria rivini shows a large pith, the circle of vascular bundles, and, below the epidermis, strands of collenchyma, alternating with assimilatory parenchyma and acting as mechanical tissue.

In the flower scapes of Papaver, Armeria maritima, Lychnis viscosa, and Anthericum ramosum the mechanical tissue is in the form of a closed ring of sclerenchyma. Between this and the epidermis occurs green tissue, while the vascular bundles are disposed within. On microscopic examination of a transverse section of the haulm of Juncus glaucus, we perceive under the epidermis green tissue and sclerenchyma bundles, which alternate with one another. We further see distributed in the ground tissue wide air-canals, and numerous vascular bundles containing some fairly wide vessels, and provided both on the inside and outside with a layer of bast fibres. The structure which we observe on examination of a cross section of the haulm of Sesleria coerulea is easy to understand, and we are here specially interested in the layers of bast in the vascular bundles, since

they play the part of mechanical tissue. In the haulm of Molinia coerulea a closed ring of mechanical tissue is present, which is further strengthened by subepidermal ribs. The vascular bundles are partly imbedded in this ring and partly surrounded by it. The haulm of the rye is similar in construction.

It is also of interest to make ourselves acquainted with the mechanical tissue of the leaf sheaths of grasses. These tubular sheaths embrace the stem part of the haulm, and their special function is to shelter the delicate growing regions of the stem, which, as is well known, are situated in grasses at the base of the internode. We prepare delicate transverse sections from the part of a rye haulm lying immediately above a young node. Below the epidermis of the inner side of the leaf sheath we perceive a tissue free from chlorophyll, but under the epidermis of the outer side, chlorophyll-containing tissue. The vascular bundles are readily recognised; they are provided both inside and outside with a well-developed layer of bast fibres.

If we wish to acquaint ourselves with the arrangement of the mechanical tissue in structures not resistant on all sides to flexion, we prepare, *e.g.*, transverse sections of the leaf of Phormium tenax and through the mid-rib of a fully developed leaf of Zea Mais. The structure of the Phormium leaf is not quite the same above and below, but at all events the stereome bundles covering the vascular bundles on the under and lower sides of the leaf at once strike the eye. In Zea subepidermal masses of sclerenchyma are present on the upper side of the leaf; the mechanical tissue of the under side of the leaf stands in exact relation to the grouping of the large vascular bundles.

As we have already remarked, rhizomes and roots, more especially, are constructed to resist tension and pressure. We prepare a transverse section through the rhizome of Carex glauca. The peripheral ring of sclerenchyma, not, it is true, very well developed, serves as protection against radial pressure. The central cylinder of wood, consisting of thick-walled elements, and in which most of the vascular bundles are scattered (a few bundles also lie outside this cylinder), serves to secure resistance to tension. We find an entirely similar arrangement of the mechanical tissue on investigation of the transverse section of lateral roots of the first order of Zea Mais. The peripheral ring of sclerenchyma is here, however, developed much more highly than in the rhizome of Carex.[1]

It may further be remarked that on treatment of the sections with phloroglucin and Hydrochloric acid, the sclerenchyma and bast fibres of the mechanical tissue, in all cases which I examined, stained red (see 42), and must therefore have been lignified.

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[1] See also the memoirs cited at the end of 195, and especially Schwendener's work.

197. Correlation.

The growth of one part of a plant frequently exerts a certain influence on that of another part of the same individual. We have in recent times begun to devote special attention to the facts relating to correlation in the vegetable kingdom, and we will now acquaint ourselves with some of these facts.

If we deprive young fir plants (Abies excelsa) of their apical shoot, it will be found that, in the course of one to three years, one or more of the horizonal lateral shoots of the uppermost whorl elevate themselves. One of these generally obtains the upper hand; it then completely replaces the terminal shoot removed. This is seen not only in its orthotropism, but also in the arrangement of its branches. A horizontal lateral shoot of the fir branches chiefly in a horizontal direction, right and left, while a normal terminal shoot or one of these substituted lateral shoots forms four- or five-rayed whorls of branches. In the experiments which I made to acquaint myself with the phenomena of correlation here under consideration, I used fir plants growing in the woods, about the height of a man.[1]

If we place potatoes in a dark place with their morphological base downwards (the tubers need not be laid in soil, and we need not even supply them with water), we shall find that after a shorter or longer time scarcely any buds sprout except those situated near the morphological apex. If, however, in some of the potatoes we now remove the shoots developing at their apex as they appear, it is seen that this operation induces the development of more basally situated buds, which would not have developed, or only to a slight extent, if we had not broken off the shoots appearing at the apex.

A further instance of correlation can easily be demonstrated in

seedlings of Phaseolus multiflorus. If we grow these in loose garden soil, and cut off the epicotyl, just above the soil, when it has reached a length of a few cm., the buds present in the axils of the cotyledons develop into shoots, in place of the organ removed, and soon appear above-ground.

It is a well-known fact that the stalks of the flower buds in almost all species of the genus Papaver, at a certain stage of development, curve downwards, and this, as far as is known, depends on correlation. The bud, in organic connection with its stalk, exerts on it a certain influence, which causes it to curve strongly and bend downwards. Proof of this is afforded by the following experiment, first performed by Vöchting,[2] and which I repeated with good results using a poppy plant growing in the open. We cut a number of buds from their stalks, lay some of them aside, and fasten others to their stalks again by means of fine silk. The curvature of the stalks always disappears after a time, and our experiment teaches that its occurrence is by no means the result simply of the weight of the bud, but depends on correlation.

It is highly instructive, in connection with our present subject to pay particular attention to the peculiarities and behaviour of the bud scales of different plants.[3] In Aesculus and Pavia, the outer scales of the winter buds are brown and membranous. Then come succulent, green, very large scales, and finally the foliage leaves. Investigation of the history of development teaches that all the bud scales are merely foliage leaves arrested at an early stage of development, and the following experiment leads to the same result. If, just after the winter buds have sprouted, we cut off the tips of Aesculus or Pavia shoots, and, without removing the shoots from the parent plant, strip off their leaves, then in the course of the summer the buds lying in the axils of the leaves develop into foliage shoots, whereas normally, they would become winter buds. The notable point about this case of correlation lies, however, in the fact that these shoots produce no bud scales, but only (at least, so I observed in my experiments) forms intermediate between scale leaves and foliage leaves, together with foliage leaves. The lower leaves of the shoot have small but still segmented laminæ, and these are seated on a green scale-like leaf structure, while the leaves at a higher level have the form of normal foliage leaves. There is thus in Aesculus and Pavia, as also in other plants, a very well marked correlation between the presence or absence of the

apex and leaves on the one hand, and the way in which the development of the buds proceeds, on the other.

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[1] See Sachs, *Lectures on Plant Physiology.*
[2] See Vöchting, *Die Bewegungen der Blüthen und Früchte*, Bonn, 1882.
[3] See Göbel, *Botan. Zeitung*, 1880, pp. 771 and 807.

V. MOVEMENTS OF VARIATION.

198. Experiments with Acacia lophanta.

Movements of variation, for the most part effected by means of special pulvini, are characteristic of many plant structures. They are due partly to internal causes, partly to the influence of external conditions (variations in illumination, shocks, etc.), but all these conditions merely induce, as we shall see further on, changes of turgor-expansion in the cells of the tissue complexes from which the movements proceed, and this is of special importance. The following experiments, firstly with Acacia lophanta, will make us accurately acquainted with these remarkable movements.

The leaflets of the compound leaves of Acacia lophanta, a plant which can be raised in flower-pots from seed, are expanded horizontally, when the plant is exposed to bright diffuse daylight. In the evening the leaflets lay themselves together in an upward direction, spreading out again on the following day, under the influence of the stimulus of light. We can, however, compel the leaflets to assume the night-position even during the day, if we take the plants into a dark place. After the lapse of from half an hour to an hour the leaflets have laid themselves together, but they expand again if the plants are anew exposed to diffuse light. The movements of the Acacia leaflets are the result of the change in the condition of illumination, for they are also exhibited, as can easily be determined, if the temperature remains constant while the observations are being conducted.

I was able to determine that the leaflets of Acacia lophanta also took up a position similar to the darkness position when the plants were exposed to direct sunlight. An Acacia with expanded leaves was placed in direct sunlight under a bell-glass. The leaflets at once laid themselves together; they again spread out horizontally, when the plant, without the removal of the bell-glass, was exposed to bright diffused daylight.

It is a very interesting fact that plants of Acacia lophanta which have been exposed to normal conditions of illumination, and are then completely removed from the action of the light by being placed in a perfectly dark place (*e.g.* a cupboard), nevertheless continue to execute the periodical movements of the leaves induced under normal conditions by the daily recurring alternation of light and darkness. A particularly vigorous plant of Acacia lophanta growing in a small flower-pot, and kept in constant darkness for four days, expanded its leaflets in the daytime, and laid them together at night; the amplitude of the movement, it is true, gradually diminished, and at the end of four days the after-effect movement completely ceased; the leaflets had become darkness-rigid, and the leaflets of the older leaves were now horizontal in position, while those of the younger leaves were more or less upwardly directed. When the plant was again exposed to normal conditions of illumination, the leaflets again became phototonic, *i.e.* they reacted anew to the alternation of day and night.

If we wish to study accurately the periodic after-effect movements executed by leaflets of Acacia plants which have first been exposed to normal conditions of environment, and then kept in constant darkness, we proceed thus, following Pfeffer. We cut out of stiff paper a number of triangles of different but known size of angle. It is sufficient if successive triangles differ from each other by an angle of say ten degrees. The triangles are held between the leaflets in order to determine their inclination towards each other, and if the observations on a particular leaf of an Acacia plant kept in darkness are repeated frequently in the course of the day (say every two hours), we shall obtain fairly accurate information as to the history of the periodic after-effect movements.[1]

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[1] See Pfeffer, *Die periodischen Bewegungen der Blattorgane*, Leipzig, 1875.

199. Experiments with Phaseolus multiflorus.

In the leaves of Phaseolus, as in those of Acacia lophanta, periodic movements are to be observed, which owe their origin to the daily recurring alternations of illumination. The main leafstalk rises in the evening and sinks in the morning, while the three leaflets (see the adjoining illustrations, Figs. 180 and

181) under the influence of light assume a nearly horizontal position,* whereas in the absence of light they lay themselves together downwards. The leaflets of Phaseolus exhibit also periodic after-effect movements, when plants which have first been growing under normal conditions are then placed in constant darkness. For observations on this point, we require vigorous pot plants. In investigations which I conducted, the periodic after-effect movements lasted several days, though certainly with diminishing amplitude. The leaves finally became darkness-rigid, in

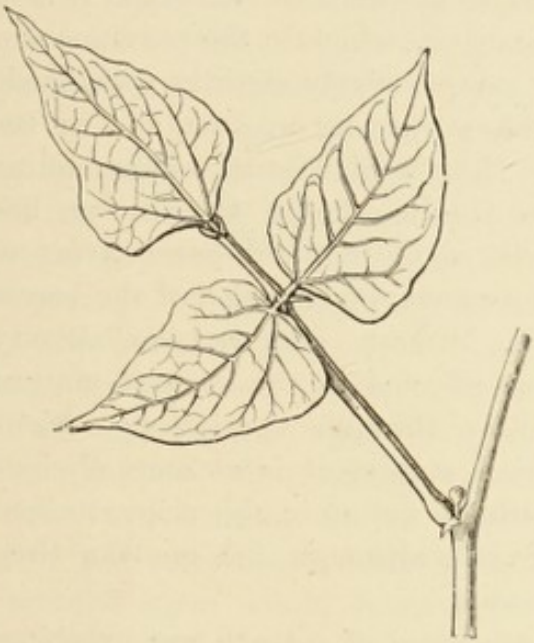

FIG. 180.—Leaf of Phaseolus multiflorus, day position.

FIG. 181.—Leaf of Phaseolus multiflorus, night position.

which condition they were expanded horizontally. Once more exposed to normal conditions, the younger eaves speedily returned to the phototonic condition; the older ones evidently reacted far less energetically to the stimulus of light.

In the production of the periodic movements of not fully-grown leaves of Phaseolus, processes of growth certainly play some part. The movements of mature leaves of Phaseolus, however, as is also the case in many other plants (Mimosa, Oxalis, etc.), are entirely due to variations in the amount of turgor-expansion. And these changes causing the movements show themselves in the joints of the leaves (the pulvinus of the main leaf-stalk as also the pulvini

* This takes place, however, only in bright diffuse light. In direct sunlight the leaflets assume, particularly at noon, a position which in some respects resembles their night position.

of the three leaflets). It is instructive to compare the anatomical structure of the joint with that of the rest of the leaf-stalk. If we examine transverse sections of the large pulvinus at the base of the main leaf-stalk of Phaseolus, it is particularly striking that below the epidermis, which is beset with hairs, occurs a very strongly developed parenchyma, whose cells are almost the same in character on the under and lower sides of the pulvinus. Fairly in the middle of the section we observe a number of vascular bundles, which surround the pith. On microscopical examination of a transverse section from the middle of the main leafstalk, it is at once seen that the tissue of the cortex, which in the pulvinus was so well developed, is here only comparatively slightly developed; it occurs as collenchyma (in the projecting angles), and as the ordinary cortical parenchyma. The vascular bundles surrounding the pith are not aggregated in the middle of the section, but lie more peripherally. The cells of the cortical parenchyma of the pulvinus bring about the movements of variation of the leaves in the bean, and also in Acacia, Mimosa, etc., and, as already remarked, these movements are effected in the case of mature organs, not by growth, but solely through varying conditions of turgidity, the truth of which statement is at once obvious when we consider that the pulvini maintain the size attained on the completion of their growth, although for months they bring about movements of the leaves.

In order to prove that no processes of growth are exhibited in connection with movements of variation, we make ink dots on one flank of the pulvinus in the manner described in 153, and repeatedly, for several days, measure the distance between them by means of a horizontal microscope. Great care must be taken that the pulvinus is always in the same position when the measurement is made.

The periodic movements of variation induced by change in the conditions of illumination are due to the fact that the cortical parenchyma of the pulvinus on two opposed sides does not vary in turgidity and change in length to the same extent. Thus, *e.g.*, the evening depression of the leaflets of a bean leaf takes place because the cells of the parenchyma of the upper side of the pulvinus turgesce more vigorously than those of the antagonistic side, so that a convex curvature of the upper side of the pulvinus is brought about.

One important factor, however, will not be obvious without the

following experiment. From a bean plant growing in a flower-pot we cut off towards evening, with a very sharp knife, the upper half of the pulvinus of the terminal leaflet of one of the trifoliate leaves. When darkness comes on, the lateral leaflets sink as usual, the terminal leaflet rises, and I found, *e.g.*, in an experiment made in July (the operation was performed at 5 p.m., and the plant was then placed in a dimly lighted place), that at 11 o'clock at night it was directed almost vertically upwards. By next morning the terminal leaflet had in my experiment sunk again. It follows from this that in leaves which exhibit movements of variation, the evening change of position does not take place because the turgidity of the cells of one only of the opposed halves of the pulvinus (in our case the upper) is intensified in darkness. On the contrary, darkness raises the turgidity of all the cells of the parenchymatous mantle of the pulvinus, while exposure to the light reduces it; but this elevation or depression of the turgidity does not proceed at the same rate in the opposed halves of the pulvinus. In the pulvini of the bean leaflets, for example, the turgidity augments more rapidly in the cells of the upper half than in those of the lower; hence the leaves bend downwards. But that the turgidity does at the same time increase in the lower half of the pulvinus is clear from our experiment, since if this had not been the case, the leaf operated upon would have been unable to raise itself in the evening.

Some further experiments may here be made which teach that the movement-joints of the bean—and those of other plants are similar in their behaviour—are geotropically sensitive.

We grow beans in flower-pots in bright diffuse light. When the plants have formed a few trifoliate leaves, we lay strips of wood over the soil in the pots to prevent it from falling out, invert the plants, and leave them in the inverted position exposed to the light (best in the light shade of a tree). It is of advantage if the plants are allowed to grow from the beginning in the light shade of a tree. If the temperature is high we can proceed to observations on the inverted plant even at the end of six to eight hours. Before inverting we had measured the angle formed with the shoot axis by the stalks of the primordial leaves, as also of the trifoliate leaves. We had likewise determined the angle made by the blades of both kind of leaves with the leaf-stalks. If we repeat these determinations after the plants have

been inverted for several hours, we find above all things that the blades have risen considerably, and a slow subsequent elevation is also exhibited in the leaf-stalks dependent on the negative geotropic irritability of the joints.[1]

If we now bring the plants back again to the upright position, the leaves regain their normal position in the course of a day. If the plants have been kept inverted for several days, and are then at last placed upright again, the leaves resume their original position very slowly or not at all, since the geotropic curvature of the pulvini has now been fixed by growth.

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[1] For further particulars see Pfeffer, *Periodische Bewegungen*, 1875, and A. Fischer, *Botan. Zeitung*, 1890.

200. Experiments with the Lever Dynamometer.

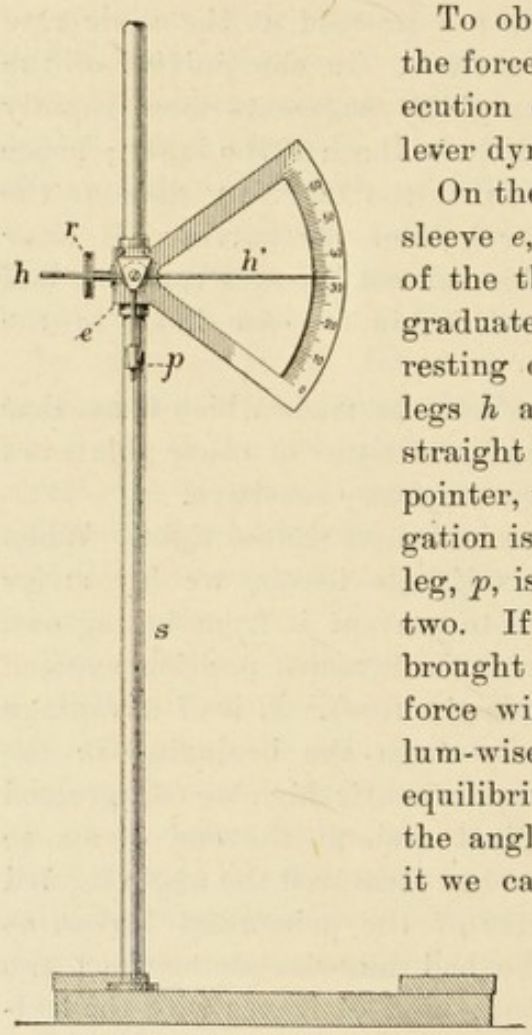

Fig. 182.—Pfeffer's Dynamometer.

To obtain an approximate value for the force which is developed in the execution of movements by pulvini, the lever dynamometer may be employed.[1]

On the brass column s is movable the sleeve e, which can be fixed by means of the thumb screw r. This carries a graduated arc, and a three-legged lever resting on a knife edge. Of the two legs h and h' which are in the same straight line, the longer serves as the pointer, while the object under investigation is laid on the shorter; the third leg, p, is at right angles with the other two. If it is displaced, *e.g.*, by pressure brought to bear on the shorter leg, the force with which it endeavours, pendulum-wise, to get back to its position of equilibrium, increases with the sine of the angle of deflection. By weighting it we can, of course, vary the force required to produce a given deflection, and so also regulate the deflection of the pointer effected by applying

a plant structure to the short leg *h*. The lever dynamometer may be obtained from Albrecht, Tübingen.

We select for examination a vigorous specimen of Phaseolus. We fix a wire along the midrib on the upper side of a primordial leaf, so that it reaches up to the pulvinus at one end, and at the other extends beyond the tip of the leaf. The wire must be such that it will not bend under the forces to be liberated. Usually, in experiments with bean leaves, a wire 60–80 mm. in length and say 0·2 gr. weight, formed by twisting together two pieces of fine iron wire, yields good results. The wire is tied to the leaf at at least three places, the thread in each case being taken round the wire and midrib. The end of the wire projecting beyond the leaf is laid on the short arm of the lever, and it is best to fix it there by means of thread or wire.

Before proceeding to the experiments it remains to be determined what relation exists between the force acting on the short leg of the lever on the one hand and the corresponding deflection on the other. If the deflection is not too great, this relation can be very exactly determined by suspending from the short leg a scale pan, and loading this with weights. The same deflection will naturally correspond with a greater or less pressure according to the loading of the vertical arm *p* (by wax balls or otherwise). One degree of deflection will correspond, *e.g.* according to circumstances, with 0·1 gr. up to even 0·3 gr. The product of the weight corresponding with the observed deflection and the length of the leaf lying on the arm of the dynamometer, and acting as the arm of a lever, gives the moment of the resultant pressure exerted by the pulvinus.

In the experiments with bean leaves the petiole must naturally be tightly fixed as far as the pulvinus. When the apparatus is carefully arranged and vibration-free, changes in the expansive force of the opposed halves of the pulvinus due to variations in brightness evoke movements of the dynamometer legs. If we place the apparatus in the dark, *e.g.* in the afternoon, the pointer may be deflected 10° in the course of a few hours. If 1° corresponds with a pressure on the short arm of 0·3 gr., and if the lever formed by the leaf was 70 mm. in length, then the 10° would indicate a moment of 210.

Bean leaves also execute autonomous movements of moderate amplitude, each swing occupying about two hours. For these the moment often lies between 10 and 25. According as these

autonomous movements are in the same direction or opposed to the paratonic and daily periodic movements will the moments of these latter be amplified or lessened. (For further particulars see Pfeffer.)

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[1] See Pfeffer, *Die periodischen Bewegungen der Blattorgane*, 1875, pp. 9 and 97.

201. Experiments with Mimosa pudica and other Plants.

The leaflets of Mimosa pudica perform movements both as the result of shock or contact and in consequence of changes in illumination, and these are effected by the pulvini of the primary and secondary leaf-stalks and of the individual leaflets. The plants may be raised from seed in flower-pots; care must be taken in cultivating them that they are exposed to a high temperature (20°–25° C.), and that they are well supplied with water. This last is best attained, in cultures carried on in an ordinary room, by covering the plants with large bell-glasses soon after they appear above ground, without of course interfering too much with the circulation of the air. A good number of seedlings may be grown in the same pot, but they are early transplanted into separate ones, and the cultivation is continued in warm moist air at the window. In vigorous plants the main leaf-stalk is during the day more or less upwardly directed, and the leaflets are expanded. If in the daytime we suddenly darken a plant, by placing over the covering bell-glass a cardboard box, the main leaf-stalk raises itself not inconsiderably, so that the angle which it makes with the stem becomes more acute, and the leaflets lay themselves together in an upward direction. If the Mimosa plants under the bell-glasses are left in the daytime, untouched, to the influence of the light, it is seen that the leaflets lay themselves together towards evening, and that the main leaf-stalks sink as darkness comes on. Thus in Mimosa pudica sudden darkening during the day on the one hand, and the normal evening darkening on the other, act in the same manner on the leaflets, but do not act in the same manner on the main leaf-stalks.[1]

If plants of Mimosa, at first cultivated under normal conditions, are exposed in a sufficiently moist atmosphere to constant darkness, they exhibit after some time, like Acacia and Phaseolus, after-effect movements of the leaves, *i.e.* the leaflets are spread out in the daytime, and laid together at night-time. Gradually these

movements cease, and the leaves are no longer able to react even to shock or contact. In observations, however, on plants of Mimosa placed in darkness, it is to be noted that leaves of different ages do not behave in the same manner. When the daily periodic after-effect movements are no longer taking place, the darkness-rigid leaves do not appear in the normal night position, but, on the contrary, the main leaf-stalk in such leaves is nearly horizontal in position, and the leaflets are outspread. If a plant is now for a short time illuminated, and then again brought into the dark, it still does not react to the change of illumination, and to shock or contact; its leaflets do not go together; the plant is still darkness-rigid. The phototonic condition only returns after long-continued illumination, and then only does the Mimosa regain its capacity of reacting to change of illumination; later still it becomes sensitive also to shock or contact. I kept one plant of Mimosa pudica, *a*, in the dark, at a temperature of about 20° C., from August 16 to half-past seven in the evening of August 21. On the evening of August 21, all the leaves up to the two youngest were completely darkness-rigid. The plant *a* was now, with another, *b*, which had been exposed to normal conditions of illumination, placed in front of a window. On August 22 the light was allowed to act on the two plants from sunrise till 10 o'clock; they were then both placed in the dark, but while the leaves of *b* closed up, those of *a* remained outspread. The leaves of the plant *a* also did not yet react to shock or contact; they were still darkness-rigid. After being in the dark for half an hour, both plants were brought back into the light again. On the evening of the 22nd August some of the leaflets of the plant *a* laid themselves together, and on the 23rd August irritability to contact returned, and also a very energetic closing movement of the leaflets took place in the evening. Many leaflets of the plant *a*, however, during the last days of the research became yellow and fell.

If plants of Trifolium pratense are observed in the open, or if we keep under observation plants raised from seed in flower-pots, we shall find that the leaflets, which during the day are outspread, lay themselves together in an upward direction in the evening. The leaflets of Oxalis Acetosella, on the contrary, fold together downwards in the evening.

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[1] As to the causes of these complicated phenomena, see Pfeffer, *Die periodischen Bewegungen*, etc., 1875, p. 74.

202. Movements of Variation in Mimosa pudica Evoked by Shock or Contact.

Mimosa pudica is only irritable to any considerable extent at a fairly high temperature, and when the soil and the surrounding air are sufficiently moist. If, however, under such conditions, plants of Mimosa raised from seed in flower-pots are agitated without the plants themselves being touched, a striking irritation-effect is exhibited. The primary leaf-stalks sink, the secondary leaf-stalks approach one another, and the leaflets lay themselves together forward and upwards (see Fig. 183). These movements are all effected by pulvini, which are situated at the base of the leaf-stalks and leaflets, and have a structure similar to those of Phaseolus leaves. We can, however, incite the Mimosa to movements, not only by shocks but also by contact. If the upper side of the large pulvinus at the base of the primary leaf-stalk is carefully touched, no movement, it is true, follows; but movement at once appears if the lower side of it is so stimulated. Accordingly only the under side of the pulvinus reacts to impact stimulus [Stossreiz]; its cells lose water, which may really pass into the intercellular spaces occurring between the cells, the hydrostatic equilibrium in the pulvinus is upset, so that finally, according to Haberlandt, the stimulus-conducting elements in the vascular bundle region also let fluid escape. Owing to the contraction of the underside of the pulvinus due to loss of water by its cells, and the co-operation of the upper side of the pulvinus which is in a state of high positive tension, an energetic downward movement of the main leaf-stalk is brought about. The loss of water by the pulvinus on stimulation, and the existence in the pulvinus of conditions of tension, may be demonstrated as follows:—

The primary leaf-stalk is separated by a sharp cut from its pulvinus, and the Mimosa is then left for some time under a bell-glass in an atmosphere saturated with moisture. If the pulvinus, after it has risen to some extent, is stimulated, it sinks, and water issues from the cut surface. In an uninjured plant the water is carried away from the pulvinus, chiefly into the stem or leaf-stalk. If we cut away one of the large pulvini close to the shoot axis, without removing it from its leaf-stalk, it bends naturally in consequence of the stimulus in the usual way. If we now by two longitudinal cuts isolate the upper and lower parenchyma of the

pulvinus from the fibrovascular system, the former vigorously curves downwards, the latter feebly upwards. If the organ of movement so prepared, and still remaining in connection with its leaf-stalk, is laid in water, the curvature of the upper parenchyma, and especially the upward curvature of the lower, since its cells have again become turgescent, is intensified. The isolated lamellæ of parenchyma, moreover, exceed the fibrovascular bodies of the pulvinus in length, and everything points to the conclusion that in uninjured motile organs considerable tensions must exist be-

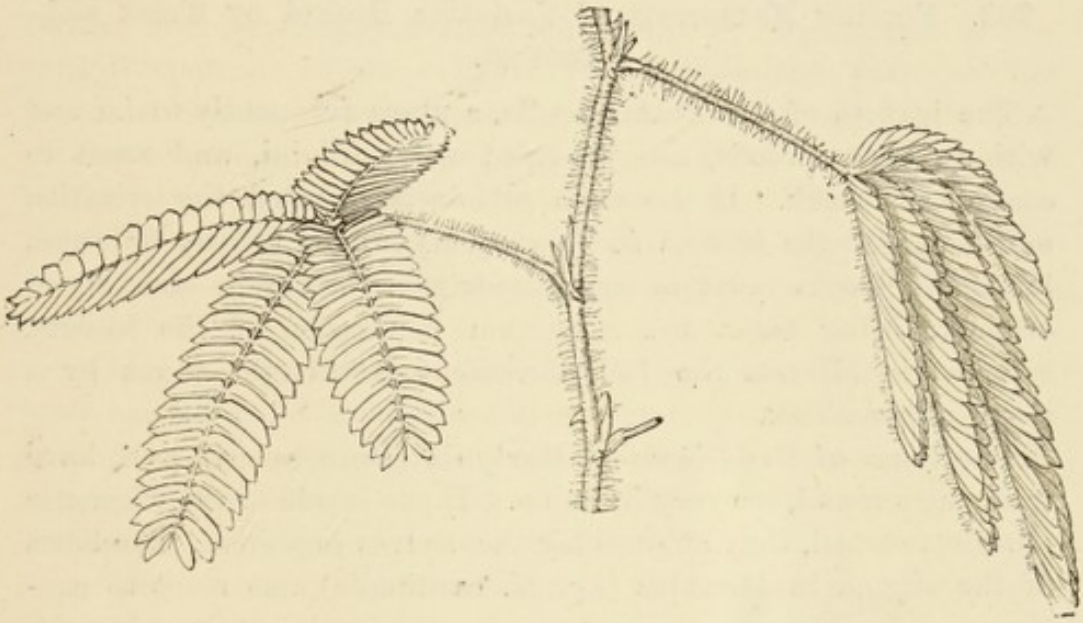

FIG. 183.—Portion of a shoot of Mimosa pudica. The leaf represented on the left is unstimulated, that on the right hand stimulated.

tween the axile strand on the one hand and the parenchyma on the other.

The following simple experiment is very instructive. We carefully, and so as not to agitate the plant, cut off with a pair of scissors one of the small leaflets at the end of a secondary leaf-stalk of a very sensitive Mimosa, or we stimulate a leaflet of a Mimosa by focussing on it convergent rays of light from the sun by means of a lens. There is now exhibited a transmission of stimulus, due to movement of water in the plant. Advancing from the tip towards the base of the secondary leaf-stalk, the transmission of stimulus causes more and more remote pairs of leaves to lay themselves together, and then the leaflets of neighbouring secondary leaf-stalks lay themselves together (first the lower and later the higher ones), and, indeed, even the main leaf-

stalk may sink and the stimulus may overflow to other leaves. When the passage of the stimulus has ceased, and no further movements are evoked, the leaves return again after a few minutes to their position of rest.[1]

---

[1] See Pfeffer, *Physiologische Untersuchungen*, 1873, and Sachs, *Lectures on Plant Physiology*. Haberlandt's contributions I do not agree with in all respects (*Das reizleitende Gewebesystem der Sinnpflanze*, Leipzig, 1890).

203. Further Movements of Variation Evoked by Shock and Contact.

The leaflets of Oxalis Acetosella, a plant frequently to be met with in damp woods, are provided with pulvini, and react to contact or shock. If the main petiole is agitated, the irritation movement of the leaflets (a depression) can be clearly followed, but many shocks must be communicated to the main leafstalk in order to bring about the maximum depression of the leaflets, whereas in Mimosa the full movement is brought about by a single slight shock.

The lobes of the stigma of Martynia (Gesneraceæ), as I have frequently found, are very irritable. If the inside of the stigmatic lobes is touched, they at once lay themselves together. The lobes of the stigma in Mimulus (*e.g.* M. cardinalis) also react to contact.

The five filaments in the Cynareæ are attached at their lower end to the corolla tube. The anthers are united to form a tube through which the style grows. When the pollen is ripe the filaments are irritable, and if not stimulated appear curved, convex side outwards. If stimulated by shock or contact they straighten, at the same time shortening. Pulvini the filaments do not possess; but the whole parenchyma surrounding the axile vascular bundle is irritable, and when subjected to stimulation loses considerably in expansive force, owing to escape of water, and so is brought about the contraction. To satisfy ourselves that the filaments of the Cynareæ are irritable, we may experiment with the flowers of Centaurea jacea. We isolate single flowers from the capitulum, cut across the corolla, filaments, and style, somewhat above the point of insertion of the stamens, and fix the separated sexual apparatus on a cork by means of a pin. It is then placed in a moist atmosphere under a bell-glass. When the preparations

have recovered, they are sensitive to stimulation. The free filaments move when touched, and after executing movements regain their irritability again under favourable external conditions in a few minutes. The movement of the free filaments is due to the fact that the side touched shortens and always first becomes concave.

204. Spontaneous Movements of Variation.

Spontaneous movements of variation are to be observed in various plants whose leaves are provided with pulvini (Mimosa, Oxâlis, Trifolium), and are brought about by fluctuations in the turgor-expansion of the cells of the antagonistic parts of the pulvinus, due to internal causes. In order to acquire information on the subject, we select for examination Trifolium pratense, and cultivate the plants from seed in a flower-pot. When the plants are fairly advanced in development it is best to reject the weakly individuals and retain for observation only a few vigorous specimens.

It has already been mentioned that leaves of Trifolium react very vigorously by movements to change in conditions of illumination. In order to exclude these movements as far as possible, we bring the flower-pot with the Trifolium plants into a dark cupboard, or place over it a cardboard box, and let the plants remain in the dark also on the next day, on which the observations proper are to begin. From time to time, say every half-hour, we determine the position of certain of the leaflets, and indeed it is best to fix their position at any time by means of a sketch. Numerous experiments which I made at a temperature of about 18° C. showed me that the leaflets of Trifolium pratense executed in the dark upward and downward movements, the amplitude of which was sometimes less, sometimes more than 90°. A few hours were necessary for the performance of a complete oscillation.

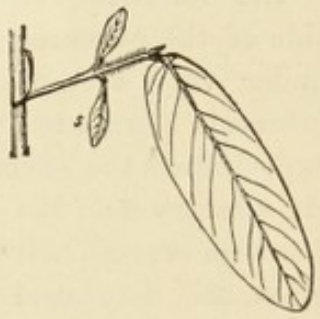

Fig. 184.—Leaf of Desmodium gyrans, reduced in size. (After Pfeffer.)

Very marked autonomous movements of variation are executed by the lateral leaflets of Desmodium gyrans. They describe elliptical paths, whose long axis is approximately parallel with the main leaf-stalk. The temperature minimum for these

movements lies at about 22° C. I saw one complete circuit made, at a high temperature, in a few minutes. The ascending journey is somewhat more slowly accomplished than the descending one (see Fig. 184).

205. The Influence of External Conditions on Some Movements of Variation.

The influence of external conditions on movements of variation has already been frequently pointed out. We may here pursue the subject further. In order to determine the influence on the pulvini of Mimosa pudica of ether or chloroform vapour, it is best in my experience to proceed as follows. A detached leaf whose leaflets have been caused by gentle stimulation to lay themselves together, is placed with its stalk in a small glass vessel containing water. The glass stands in a dish into which we have poured some ether or chloroform. The whole is covered with a bell-glass, and exposed to direct sunlight. During the narcosis, which is continued for a few minutes, the leaflets completely expand; they now react neither to shock nor contact. They recover their irritability, however, when transferred to a moist atmosphere free from chloroform or ether.

The following experiment is very instructive. The under side of the pulvinus on the main leaf-stalk of a Mimosa growing under very favourable conditions is repeatedly and at short intervals stimulated by means of a small rod of wood. (In my experiments the stimulation took place every half-minute for five or six minutes, and the under side of the pulvinus received on each occasion several blows.) If the plant is left to itself under a bell-glass, the depressed main leaf-stalk rises after a few minutes, but still it does not now react to contact. The organ of movement thus, by often repeated contact, rendered insensitive to further stimulation, again becomes irritable, however, after a short time. It is of special interest that the Mimosa leaflets outspread after narcosis with ether or chloroform, and the main leaf-stalks of Mimosa elevated after the cessation of shocks, are not irritable immediately, but only after lapse of time. It teaches, viz., that the causes on which depends the return of the structures to a condition of sensitiveness to stimulation on the one hand, and those on the other on which depends the irritability itself cannot be entirely the same.

If the soil in which a Mimosa is rooted is not watered for a long time, the plant being however exposed otherwise to normal conditions, the capacity of the leaflets for response to shock or contact falls off more and more. In an experiment made by me at about 20° C., complete loss of sensitiveness to contact appeared after four days. The leaflets in a state of dryness-rigor are outspread, but by no means yet appear limp, and they regain their irritability when the soil is again well supplied with water.

If a Mimosa is exposed for a few hours to a temperature below 15° C. (*e.g.* 10° C.), the plant passes into a state of transitory cold-rigor. The sensitiveness to contact and change of illumination disappears. The normal state returns, however, when the plant is brought back to a place at a temperature above 15° C.

To bring Mimosas into a state of temporary heat-rigor, we place the plants in a suitable thermostat at a temperature of 40° C. After about an hour (at 45° C. in a much shorter time) heat-rigor sets in. The leaflets have in presence of light laid themselves together upwards. With favourable conditions of temperature, the normal state is regained in the course of a few hours.[1]

---

[1] See especially Claude Bernard, *Leçons s. l. phénomènes d. l. vie*, 1878 Sachs, *Flora*, 1863 ; and Pfeffer, *Physiol. Untersuchungen*, 1873.

APPENDIX I.

FIRMS SUPPLYING APPARATUS, UTENSILS, ETC.

(Only the most important apparatus, etc., is mentioned.)

Seed and plant material: HAAGE and SCHMIDT, Erfurt.

Pressed yeast: VERSUCHS- UND LEHRBRAUEREI zu Berlin, See- und Torfstr.-Ecke. Also pure yeast cultures in test tubes, price 10 mk. In many cases it is sufficient to purify the yeast oneself. Yeast from a brewery is distributed in distilled water which has been sterilised by boiling. When the larger particles floating in the fluid have settled, we pour off the fluid in which the yeast cells are suspended, and put it in a cool place. When the bulk of the yeast has settled, we pour off the turbid fluid, distribute the sediment once more in water, and decant as before, proceeding in this way say four times more. The purified yeast is now again distributed in water; some is allowed to settle, and we then remove by means of a pipette a known volume of the yeast-containing fluid. Instead of the water last used, we may, under some circumstances, employ a certain quantity of a food solution (Pasteur's food solution, beer wort, etc.) appropriate for yeast cultures. If it is required to provide various portions of food solution with precisely the same quantities of yeast, it is well to proceed as follows: Water or food solution is supplied with purified yeast, as above, and then a quantity of the fluid is transferred to a further quantity of water or food solution, the final samples being removed by means of a pipette from this fluid, which is kept well shaken. In many cases it is necessary first to sterilise the water or food solution, in which yeast is to be distributed, by boiling in a flask plugged with cotton wool.

Chemicals of all kinds are to be obtained from the chemical works of MERCK, Darmstadt.

Pigments for microscopy: Dr. G. GRÜBLER, Bayrische Strasse, Leipzig.

Filter paper, ordinary and Swedish: Dr. H. GEISSLER NACHF. F. MÜLLER, Bonn.

Corks, indiarubber bungs, indiarubber tubing, etc., are to be obtained from the rubber works of WALLACH, Cassel. Rubber goods should be kept in a large closed glass cylinder, in which is a small glass half filled with turpentine. Preserved in this way, they do not become brittle and full of cracks. Rubber articles which have got hard can be rendered serviceable again by exposure for some time to the influence of chloroform vapour. They may be placed for the purpose in a closed glass cylinder, together with a glass half filled with chloroform.

Rubber corks and tubing of exceptionally good quality, made from Para rubber, are supplied by BENDER and HOBEIN, München, Gabelsberger Str. 76*a*.

Platinum crucibles, etc.: HERÄUS, Hanau.

Horn and porcelain apparatus of all kinds: EPHRAIM GREINER, Stützerbach, Thuringia.

Glass goods of all kinds (tubes, beakers funnels, etc.): ALT EBERHARDT und JÄGER, Ilmenau, Thuringia, and EPHRAIM GREINER, Stützerbach, Thuringia.

Burettes, pipettes, wash-bottles, gas preparation apparatus, etc.: Dr. R. MUENCKE, Berlin, N.W., Luisenstrasse 58.

Mills for grinding seeds, bark, etc.: GRUSON, Magdeburg-Buckau, or WENDEROTH, Cassel.

Presses and straining cloths: G. WENDEROTH, Cassel, and GEISSLER NACHF. F. MÜLLER, Bonn.

Specially good syphon barometers are supplied by GEISSLER NACHF. F. MÜLLER, Bonn. The instrument may be fixed on a stand, by means of which, when not in use, it can be inclined. The glass is thus always kept clean in the region where the readings take place.

Reservoir barometers of specially good quality, with stand and thermometer, are supplied for 32 mks., by W. HAAK, glass-blower, Jena.

Stands of all kinds are to be obtained from DESAGA, Heidelberg. Stands having a porcelain base, and specially suitable for immersion in water, are supplied by BÜHLER, Tübingen.

Apparatus for Nitrogen determinations after Kjeldahl's method: ALT EBERHARDT und JÄGER, Ilmenau, Thuringia.

Mirror galvanometers are to be obtained from the mechanical workshops of Dr. TH. EDELMANN, München, and H. MEYER, Zürich. The latter firm supplies somewhat cheaper, but still very sensitive apparatus. The mirror galvanometers, after Hermann, turned out by PLATH, Potsdam, are also specially to be recommended. The instrument, provided with two coils of 30,000 turns in all, two thermocoils of 100 turns each, excellent damping arrangement, telescope, and scale. costs 485 mks.

Spectral apparatus of various kinds are to be obtained of GEISSLER NACHF. F. MÜLLER, Bonn. A large Hofmann spectroscope, with comparison prism and measuring arrangement, costs 240 mks. A. KRÜSS, Hamburg, also supplies very good instruments. Very satisfactory pocket spectroscopes, provided with a scale, are to be obtained for 60 mks. from SCHMIDT and HAENSCH, Berlin, Stallschreiberstrasse 4.

Air pumps, suction pumps, etc., are to be obtained from Dr. MUENCKE, Berlin. The Arzberger water current pump, by means of which very complete exhaustion may be attained, is specially to be recommended. It can be obtained for 30 mks. from C. GERHARDT, in Bonn; and is supplied also by Dr. MUENCKE, Berlin; and of the highest quality by PAUL BÖHME, Brünn. I further recommend very strongly the water current pump supplied by HILDEBRAND, glass-blower, Erlangen. The small apparatus (price 2 mks.) is fixed by means of very thick rubber tubing to the water tap. The exhaustion is very complete, approaching the tension of aqueous vapour.

Balances for analytical work, to carry 100–200 gr., and turn to $\frac{1}{10}$ of a mgr., are to be obtained of G. KERN, Ebingen (Würtemburg), and P. BUNGE, Hamburg (Eilbeck). Price, 200–300 mks.

Small water motors, to be connected up directly with the water supply, and particularly suitable, *e.g.*, for setting centrifugal apparatus in motion, are to be obtained of F. A. HERBERTZ, Cologne.

Thermometers:—GREINER and FRIEDRICHS, Stützerbach; EPHRAIM GREINER, Stützerbach; KIRCHLER, Ilmenau; HAAK, glass-blower, Jena; and GEISSLER NACHF. F. MÜLLER, Bonn. For exact work it is essential to have the thermometer tested. A normal thermometer made by HAAK, Jena, gave the following results: At 0° C.—0·10°, at 10° ±0·00°, at 20°+0·05°, at 30°+0·05°, at 40°±0·00°, at 50°+0·05°. The negative sign indicates that the corresponding value is to be subtracted from that directly observed. The corrections are true on the assumption that the thread of mercury is throughout its length at the temperature to be determined. If a part of the thread projects outside the space whose temperature is to be ascertained, an addition must be made to the temperature read off and corrected from the table of errors. The correction may be calculated from the following formula:—

$$\frac{n\,(t-t')}{6500}$$

Where (n) is the length of the projecting part of the mercurial thread expressed in degrees, (t) the temperature to be measured, and (t') the mean temperature of the projecting thread. It is in most cases sufficient to put for t' the temperature of the laboratory. If this temperature is lower than the temperature to be measured, the value found must be added to the temperature indicated by the thermometer.

Evaporating apparatus, apparatus for the distillation of water and for heating drying chambers, can be obtained in all sizes from Dr. MUENCKE, Berlin.

Koch's steam sterilising apparatus, made entirely of copper, and 1 metre in height, is to be obtained from H. ROHRBECK, Berlin, N.W., Karlstrasse 24, for 60–80 mks. *See* price list of 1891–2, p. 21. Particulars as to the construction and manipulation of the apparatus are given in the catalogue.

Microscopes of excellent quality are to be obtained of C. ZEISS, Jena, and of W. and H. SEIBERT, Wetzlar. A Zeiss microscope quite sufficient for ordinary purposes is the following:—Stand No. VIIa, with eyepieces 2, 4, and 5, and objectives B and D. Price 153 mks. Magnification 95–580. Drawing prism, 20–30 mks.; the new drawing apparatus of Abbe, which permits drawing upon a horizontal surface, 60 mks.; objective micrometer, 10 mks.

A very satisfactory centrifugal apparatus, made after my designs (*see* Fig. 155), is to be obtained, at a price of 60 mks., from G. TEGTMEYER, mechanician at the Physical Institute, Jena. G. TEGTMEYER also supplies my apparatus for determining the extensibility, elasticity, and rigidity of plant structures, price 35 mks (*see* Fig. 179), as also the apparatus devised by me and depicted in Fig. 49 and Fig. 146.

From E. ALBRECHT, University Mechanician, Tübingen, may be obtained the following:—

Large clinostats, after Pfeffer, 320 mks.
Small clinostats, 220 mks.
Auxanometer, after Pfeffer, 320 mks.
Arc indicator, after Sachs, 60 mks.
Baranetzky's apparatus for registering the flow of sap, 100 mks.
Horizontal measuring microscope, 125 mks.
Lever dynamometer, 15 mks.
Gas chamber, after Engelmann, 15 mks.
Two-mirror heliostat, after Reusch, 335 mks.

Wortmann's clinostat, with all attachments, is to be obtained from UNGERER BROS., Strassburg; price 200 mks.

Azotometer: EHRHARDT and METZGER, Darmstadt; price about 30 mks.

Thermostats, thermo-regulators, gas pressure regulators, and gas burners, are supplied by Dr. H. ROHRBECK, Berlin, Karlstrasse 24. The thermostats specified under 114 and 129 of the 1891–2 price list are specially to be recommended. Price according to size, 50–200 mks. and 20–30 mks. respectively. I recommend the thermo-regulator No. 149; price, filled and tested, 19 mks. The thermo-regulators (filled with mercury and amyl alcohol), made by HAAK, glass-blower, Jena, are also very satisfactory. Price 8–10 mks.

Millimetre paper (transparent tracing paper) is to be obtained of SCHLEICHER and SCHÜLL, Düren, Rheinpreussen. Price for 25 sheets, 12 mks.

Polar planimeter: J. KERN, Aarau, Switzerland. Price, with directions for use, 45 mks.

All the apparatus mentioned in this book can be obtained from the mechanical works of DESAGA, Heidelberg. This firm has specially undertaken, either to manufacture the apparatus themselves, or procure it from the makers.

APPENDIX II.

In 84 the problem of the movement of water in the wood was discussed. We saw that the water moves in the lumina of the conducting wood elements, and if we now go into the matter a little further, it is particularly because Askenasy [1] has recently published a short treatise, which, in connection with the important work of Strasburger, certainly appears to be of great significance.

Sachs [2] has already stated emphatically that the root pressure can by no means serve to cover the loss of water experienced by vigorously transpiring plants. It is true that in the intact plant water can be raised by root pressure in larger quantities and to a greater height than in a decapitated plant, since the atmospheric pressure acts on the cut surface in the latter, while in the former the rise of the water cannot be affected by the atmospheric pressure. But for all that, even in the uninjured plant, root pressure by no means plays a part in the movement of water in the wood of strongly transpiring plants, and Hansen [3] showed that even plants with dead roots, which therefore could exhibit absolutely no root pressure at all, still actively transpire. Pot plants of Nicotiana or Helianthus, which bear 6–10 large leaves, are placed, after the soil in the pots has been soaked with water, in double-walled sheet zinc receivers, resting on tripods. The space between the walls of the receivers is filled with water. We place thermometers in the soil, cover the pots with thick card-board covers, halved and cut out in the middle so as to fit round the stems of the plants, and then heat the water. The roots are exposed for an hour or two to a temperature of 70° C. They are then, as may be established by microscopical examination, quite dead. We now carefully wrap the pots in tinfoil, cover the surface of the soil and the stems of the plants with tinfoil, and can then by repeatedly weighing prove that the aerial parts of the plants, if the temperature of the air is not too low, continue for days to lose considerable quantities of water without withering. The dead roots must absorb water from the soil.

Capillary forces may be of importance in retaining water in the tracheal channels, but, as Strasburger [4] convincingly proved, they play hardly any part in the actual elevation of the water. Nor can we call to our help the atmospheric pressure when we are considering the elevation of water to great heights (*e.g.* 200–300 ft.), and so one seems obliged to have recourse to the co-operation of the living cells of the wood.

I may here, however, direct special attention to certain investigations of Strasburger (l.c. p. 607), which it is true I have unfortunately not yet myself repeated, but the results of which undoubtedly appear to merit consideration.

Slender, compact trees of Acerplatanoides, Fagus, Pinus Laricio, or Abies excelsa, are sawn through at the base, somewhat obliquely, and under a strong current of water, which must be directed towards the cut surface. The trees, say 20 m. in height, are first left standing for half an hour in water; they are then raised by means of pulleys, and the cut surface after being smoothed is placed in contact with 5–10 p.c. Copper Sulphate solution.

The trees with which Strasburger experimented, in the course of several weeks or months, and under favourable conditions for transpiration, took up large quantities of the Copper solution. It rose, as could easily be proved, to the tops of the trees, so that the leaves gradually died, and the wood of the stems became more or less completely saturated with Copper salt. Here then the ascent of the water cannot have been caused by the osmotic activity of living cells of the wood, since the Copper salt would of course kill these cells when it came in contact with them.

Bearing in mind these experiments with large plants, interest attaches also to the results of experiments with small objects in which a portion of the channel of conduction has been killed. If, by immersion in boiling water for half an hour or an hour, we kill the lower part of branches of Populus or Salix to a length of say 20 cm., their upper leafy parts remaining uninjured, and then place the branches in eosin solution, they still suck up in course of time large quantities of fluid. Similarly if shoots are placed, immediately after being cut, with their base in 5 p.c. Copper Sulphate solution, the fluid rises to a considerable height in them, although the salt kills the living elements of the wood.

To understand the movement of water in the plant, it is important to remember that the channels of conduction in the wood, as they are developed, *i.e.*, from the time of germination onwards, fill with water. Hence the peripheral regions of the wood, in their tracheal elements, contain for the most part, even at times of vigorous transpiration, only water and no air, while of course the central elements of the wood may be devoid of water. Radial sections of stems or branches of Abietineæ are very suitable for examination.[5] The sections should be 1–2 cm. in length and of such a thickness that at least one layer of tracheides remains unopened. Investigation of the sections teaches that in general the aqueous contents of the elements falls off in quantity from without inwards.[6]

When no transpiration is taking place, the water is retained in the channels of conduction by the adhesion which the wood substance exerts on it, and by the cohesion of the particles of the water, which—and Askenasy lays very special stress on this—is exceedingly great, and no severance of the water threads takes place.

When now the sun's heat brings about transpiration, and the leaf cells lose water, they endeavour to replace the loss. They withdraw fluid from the channels of conduction in the vascular bundles; they exert a tension on the water in the tracheal channels, which, owing to the great cohesion of the water particles, is transmitted right into the roots, and there gives rise to renewed absorption of water. These views of Askenasy as to the nature of the movement of water in the wood certainly merit attention; the chief stress is always to be laid on the magnitude of the cohesive forces which the molecules of water exert on each other.

Askenasy's theory may also be maintained, as is more nearly shown in his treatise, on the assumption that the tracheal channels contain not only water, but a moderate amount of low tension air. If, however, much air penetrates into the channels of conduction, *e.g.*, when structures are cut off in the air, then a rapid passage of water into the objects is rendered impossible, and they wither, although placed with the cut surface in water.

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[1] See Askenasy, *Verhandlungen d. naturhistorisch-med. Vereins zu Heidelberg*, N.F., B. 5.
[2] See Sachs, *Lectures on Plant Physiology*.
[3] See Hansen, *Arbeiten d. botan. Instituts in Würzburg*, B. 3, p. 312.
[4] See Strasburger, *Bau und Verrichtung der Leitungsbahnen in den Pflanzen*, 1891, p. 808.
[5] See Russow, *Botan. Centralblatt*, 1883, B. 13, p. 101, and Strasburger, *Leitungsbahnen*, p. 685.
[6] On the quantity of air in the wood, see Strasburger, *Leitungsbahnen*, p. 682.

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