PLANT PHYSIOLOGY

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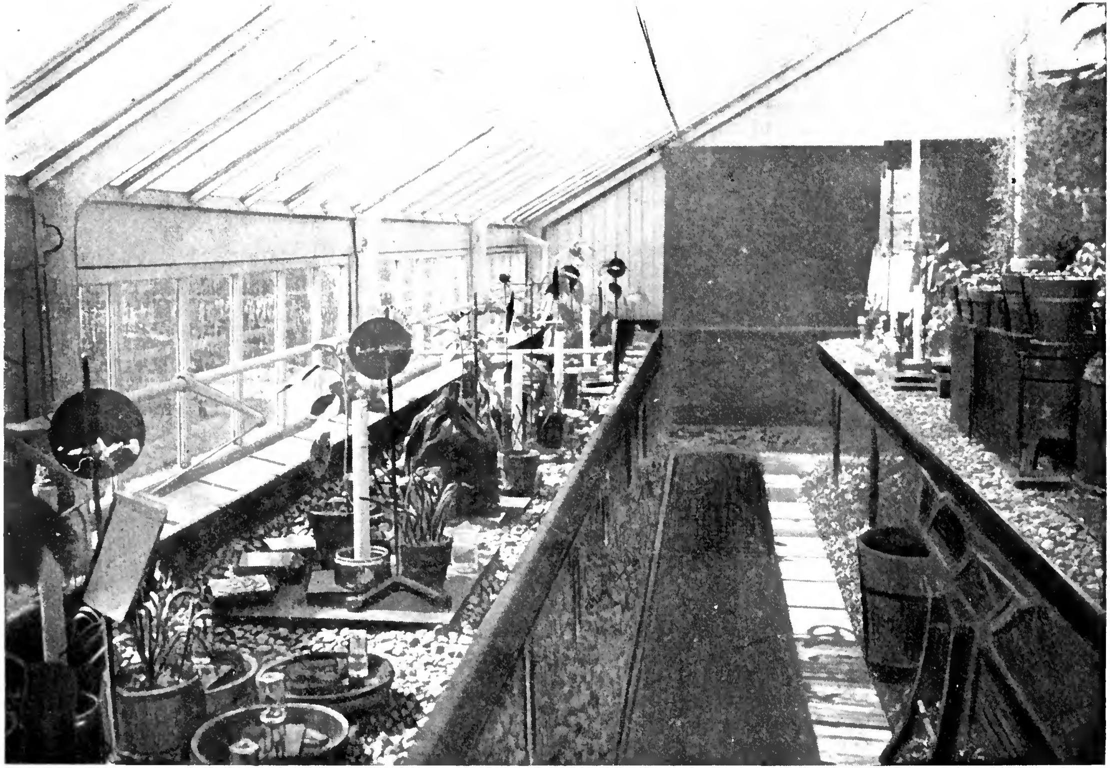

View in an Experiment House.

Frontispiece.

A LABORATORY COURSE

IN

PLANT PHYSIOLOGY

ESPECIALLY AS A BASIS FOR ECOLOGY

BY

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PREFACE.

IF the present book is found to have any merit in its particular field, it will consist chiefly in its practicability. With but few and clearly indicated exceptions, everything recommended in it has been repeatedly tested and found effective both by my students and myself. The book is the resultant of six years of effort and experiment directed to this end with classes of from eight to twelve students.

The illustrations of the experiments are in every case from photographs of experiments in successful operation. Such pictures, while inferior in clearness of detail and perhaps in artistic effect to good drawings, have at least this conspicuous merit, that they may be accepted and followed with perfect confidence.

In developing the course and the experiments herein recommended, I have tried to utilize, and, naturally, to improve upon, the best work done by others in the same lines. Little account is taken in these pages of the exact appliances and methods with which the classical work of plant physiology has been done, for the investigation phases of the subject lie without the scope of this work, and they are, moreover, summarized admirably in Pfeffer's great Handbook. But all phases of plant physiology utilizable for purposes of general education are meant to be included. The systematic attempt to simplify physiological methods and appliances for student use began, I believe, with Detmer's "Praktikum," in 1887, was continued in Hansen's "Physiologie" and Schleichert's "Beobachtungen und Experimenten" in 1890, in Oels's

"Versuche" of 1893, in MacDougal's "Experimental Physiology" in 1895, and in Darwin and Acton's "Practical Physiology" in 1896. Several of these works have passed to later editions, and to translations. A number of contributions to the simplification of appliances have appeared in the Botanical Gazette, notably by Arthur (in **22**, 463), by Barnes (in **12**, 150), by Copeland (in **26**, 343; **29**, 347, 437), by Stevens (in **20**, 89), by Stone (in **17**, 105; **22**, 258), by the present writer (in **27**, 255), and by others, all of which are mentioned in their appropriate places in the following pages. To these should be added Hansen's papers in Flora, **84**, 352 and **86**, 469. I have made much use also of two small works, which, not having been regularly published, are not included in the bibliography on a later page, namely, Arthur's "Laboratory Exercises in Vegetable Physiology" (Lafayette, Indiana, Kimmel & Herbert, 1897), and MacDougal's "Plant Physiology: Directions for Practical Work" (privately printed, Minneapolis, 1897).

It is my pleasant duty to state here that the book has the great advantage of having been read and criticised in manuscript by Professor Charles R. Barnes of the University of Chicago, to whom I make my grateful acknowledgment. Most of Professor Barnes's suggestions are embodied in the following pages, but he must not be held responsible for approval of all in the book, since in places where we differed in opinion I have followed my own inclinations, perhaps to the detriment of the work. I have also to thank Mr. E. J. Canning, Head Gardener at Smith College, for his constant and skilled assistance in everything relating to the cultivation of materials for the course.

CONTENTS.

PART I.

ON THE METHODS OF STUDY IN, AND THE NECESSARY EQUIPMENT FOR, A COURSE IN EXPERIMENTAL PLANT PHYSIOLOGY.

1. INTRODUCTION.

THIS book has a twofold purpose. First, and especially, it aims to be a guide to a practicable, truly proportioned, logically complete elementary laboratory course in the leading facts and principles of plant physiology. Second, it seeks to provide a handbook of information upon simple physiological experimentation for the use of those who may have any interest in that subject. In both these directions it tries to bring together the best of what is already known, and to add thereto some new suggestions. It is not intended to give physiological information, but to serve as a guide to the acquisition of a general physiological education.

It is well to note at the outset what place such a course as is here outlined may be expected to hold in botanical education at the present day. To those in touch with contemporary botanical progress the answer will be plain. The present movement in the science, both in investigation and in education, is strongly towards its dynamical phases,—towards the investigation of the "vital" phenomena of plants (Physiology proper), and towards the elucidation of the factors determining plant structure and distribution (Ecology). This movement is not only manifest in purely scientific study, but is equally plain in economic investigations. It is especially marked in the study of plant diseases (Pathology) in the Experiment Stations, where a knowledge of pure physiology is coming more and more to be recognized as indispensable to successful pathological investigation. Moreover, experience seems to be showing that the physiological groundwork of a general biological education may be obtained best from plants, most of whose physiological phenomena are essentially identical with those of animals, while vastly simpler to investigate and much more practicable to experiment upon. The necessity for a thorough grounding in plant physiology as an integral part of a present-day botanical education, as a basis for physiological investigation and for pathology, perhaps too as a factor in biological training, is so rapidly becoming recognized that soon no institution of the first rank, aiming to put its students in touch with present conditions and progress, will lack thorough practical courses in plant physiology. There is, however, yet another and very important reason why pure physiology is destined to greater expansion in the immediate future, namely, that it is absolutely prerequisite to the successful understanding and cultivation of the most attractive, promising, and widely interesting field now opening up to botanists and educators alike, that of Ecology. Any one who is following present-day ecological work cannot but be impressed by the fact that the best of it rests upon an exact physiological basis (as witness Schimper's admirable new work "Pflanzengeographie auf *physiologischer* Grundlage"), while that which does not is mostly of lesser worth and but tentative. For, in its last analysis, what is Ecology, the science of adaptation, other than the elucidation of the nature of the connection existing between the physiological properties and powers of the plant on the one hand, and the physical properties and processes of its environment on the other? More than one recent writer has described ecology as at present mostly a series of guesses; and so will much of it continue to be until given logical precision and a firm foundation in exact physiology.

It will be evident to any physiologist examining the course here outlined that the methods and appliances are sometimes comparatively crude. Hence they may seem to entail inexact results. But it is to be remembered that in elementary or general courses in physiology, as in elementary courses in other subjects, it is mainly qualitative results that are of value, while precise quantitative results, although the only logical end for the investigator, would be quite lost upon the beginner. This has long been recognized in Physics, where great simplification of methods and appliances has been effected to the decided profit of the educational interests of the subject. It is not, however, to be inferred that, since it is the qualitative rather than the quantitative aspect of the results that is valuable in such a course as this, therefore quantitative methods are not those to be employed. On the contrary, the exact quantitative method and spirit are scientifically and educationally the best, one may even say the only permissible, for even qualitative work; and it is entirely in this method and spirit that the present course is intended to be carried on. Simple, even crude, though some of these appliances are, there is no one of them which, correctly used, gives other than the correct *kinds* of results, and usually with approximate accuracy.

In most minds the practical study of plant physiology is associated with a necessity for the possession of the many and costly appliances developed by investigators for securing precision in quantitative researches. In my own experience, however, which is confirmed by the testimony of other teachers, I have found that not only are such appliances quite unnecessary for sound physiological education, but they are, owing to difficulties of manipulation and related reasons, actually no better than the simpler though less exact apparatus, even if they are equal to them. For investigation, in which one can afford to use nothing less than the very best, the elaborate pieces are usually necessary; it is also an advantage in elementary courses to have some of them at hand for illustration and occasional demonstration; but further than this they may be entirely dispensed with. There has been for some time a movement towards this simplification of apparatus and methods, the leading contributors to which are mentioned in the Preface, but much still remains to be done. No doubt the subject will gradually work itself out in the form of a series of standard simple appliances giving results of fair accuracy, and purchasable from the supply companies at a reasonable cost. There is here opened up to the proper combination of physiological knowledge and mechanical ingenuity an attractive field for investigation in the invention of less expensive, more manageable, and more logically conclusive experiments for demonstrating the fundamental facts and principles of physiology.

The great danger in the simplification of apparatus is that it will become so crude as to introduce merely mechanical errors, or fail to ensure logical conclusiveness in results. Since a great part of the value of such a course consists in a training in the experimental habit, the greatest care should be taken to safeguard the logical quality, as well as the quantitative correctness, of the experiments, and none should be admitted which do not fairly meet these requirements.

It may also appear that some of the experiments here recommended are of too elementary a character for this course, and are such as the students must have tried, or at least have seen tried, earlier in their studies. It seems best, however, to make the course complete in itself. Repetition has its advantages, and even the simplest experiments take on a new meaning when tried by the students themselves in their proper connections; while in any case it is always possible for the teacher to replace the simpler by more complex and exact ones.

The general belief that elaborate apparatus is necessary for good student work in plant physiology is of course the reason for the persistence in places of the older method of conducting physiological courses. This method, in its extreme, is that of providing the laboratory with a piece of each of the approved purchasable kinds of apparatus, and assigning to each student a distinct topic for thorough study; the topics are usually changed two or three times in the term, and each student is supposed to keep in touch with, and to profit by, the work of his classmates. After experience of this individual system as a student, and for several years as a teacher, I am convinced that it is on the average far inferior to the collective system here advocated (in which all students of a class work together on the same topics, each trying for himself the full set of experiments), which I have now tried for five years. Of course it is only the use of simple home-made apparatus which allows this plan at all, for no laboratory could afford enough of the exact apparatus to supply every student with all pieces; but, happily, as already stated, the simpler apparatus is for beginners educationally equal to the more elaborate, if not superior to it. Of course the spirit of this system must be observed rather than the letter, and there are times when practical considerations make it profitable to have students work together in pairs (or larger groups), or when some particular experiment may best be prepared by one for the use of all. The objections to the individual or special-problem method are, that to keep in touch with so many lines of work at once entails great and needless labor upon the teacher; that students are found to derive comparatively little good from the observation of experiments not tried by themselves; that the members of the class cannot compare results and profit by the discussion thereby suggested; that they cannot derive full advantage from lectures or other theoretical instruction when this is dissociated from the subjects they are studying, as must usually be the case for most of the students on this plan; and, finally, that as no student can cover all the topics, his training and knowledge at the end of the year are badly proportioned, his more thorough knowledge of some things not compensating for his ignorance of others. On the other hand, the collective system, keeping the students all working together on the same problems, permits more profitable use of the teacher's energy; allows all students to do all of the work; lets them profit by comparison and discussion of one another's results; enables them to understand lectures which can be fitted exactly to their stage of progress; and gives them at the end of the year a well-proportioned knowledge of the entire subject. This is the principle of the method universally adopted in other student courses, and the use of the simple appliances will make it equally practicable, as it certainly is equally profitable, in physiology.

Very important in such a course as this is the proportioning of its topics. The course should aim to give a knowledge not only of the leading physiological facts, but also of the relative importance of those facts in moulding the plant kingdom as it is. Consequently, important topics should receive due attention and experimentation, even though this may be difficult and expensive; and in cases where, as in many chemical questions, no experimentation at all is practicable, the topics should nevertheless be introduced with due emphasis into their proper places in the scheme and given book- and lecture-study. Such non-practical study of occasional topics closely yoked to topics practically studied, is vastly better than no study of them at all. On the other hand, in the case of some topics of minor value, many simple and beautiful experiments are available, and the temptation to dwell upon and use many of these must be resisted, only enough being chosen to emphasize the topic in its true proportions. It is chiefly by the amount of emphasis laid upon them in the outline and lectures of his course that the student infers the relative importance of the parts of his subject. The worth of a physiological practicum, therefore, is by no means to be judged alone from its number of practicable and logical experiments, but rather by the degree of truthfulness with which it reflects to the mind of the student the true proportions of the entire subject.

We have next to consider what position such a course may best hold in a college curriculum. Theoretically it should come as early as possible, even preceding the study of structure, on which it throws a flood of light. But, as every teacher knows, what is fairest in theory often plays false in practice. The facts and phenomena with which physiology deals are of so unfamiliar and abstract a sort that the student must have a large foundation in concrete facts before he is prepared to grasp their real significance and value. A good guide in such cases as this is the law of Biogenesis, which teaches that the individual, recapitulating as he does in his own mental development the mental evolution of the race, may most naturally and advantageously acquire his knowledge in the same order in which the race has acquired it. In the development of Botany, physiology did not come first but last, and it followed after the study of structure and classification. This does not at all mean that all physiology is to be postponed to the end of the college course, but simply that it should come there as a distinct unified study, and also that whatever parts of it are introduced earlier should be made to follow and depend upon concrete structural study. The undergraduate course in Botany which seems to me to give the optimum of advantages is the following:

The first year,—a general course, arranged to give a synopsis of the subject to those who follow it no farther, and a foundation for higher work to those who do. I have tried to outline such a course in my "Teaching Botanist." *

The second year,—a course in morphology with correlated ecology, tracing the morphological characteristics and relationships of the groups from the lower Algæ to the Phanerogams.

The third year,—a course in cellular anatomy, particularly of the higher plants, with cytology and embryology.

The fourth year,—a practicum in physiology, on the principle of that here outlined.

It may be objected that such a course gives too much routine work, and that after two, or at most three, years of regular course work the students had better be given some original problem in which their powers of investigation may be cultivated. Aside from the great inherent difficulty of securing investigation from undergraduates, it is also a fact that the validity of this objection depends upon badness in the quality of the teaching; for if the teaching be of the right sort, then all

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* Published by the Macmillan Co., N. Y., 1899.

of the courses of the four years will be, from the student's point of view, investigation. There is not in the teaching of any science any successful leading method other than induction, which is the soul of scientific investigation. From the very first week of the elementary course, all of the work of all of the courses should be a series of subjectively original investigations, in which each new thing is brought before the student as a problem to be solved through proper inductive processes by his own efforts, aided by wise advice and criticism. Thus the investigation spirit should grow throughout the student's course, while at the same time he is obtaining a truly proportioned and fairly complete knowledge and training in the principal divisions of the science, giving him the very best preparation for real (or objective) investigation in the university. Again, it may seem that the present course is too inelastic, too mechanical for the good of advanced students. But it must be remembered that the great majority of students are best treated (as they prefer to be) by a rather rigid system of drill, in which definiteness, decision, and authority are predominant. The best system is that which, while rigid enough to secure the drill of the ordinary run of humanity, is yet elastic enough not to hamper the evolution of the occasional genius.

The theory of the construction of the present course is in brief as follows: It aims to secure the best return in amount and proportion of physiological training and knowledge, for the time and energy that can be given to it by the student, taking account of the many practical difficulties of cost of materials, difficulty of manipulation, length and arrangement of the college year, etc. It is a study in educational economy, and aims to secure a harmonic optimum. Naturally this optimum must be largely subjective, and other teachers will find it somewhat differently. Since all physiological operations have their seat in protoplasm, the course begins with a study of its structure and properties. The physiological phenomena of living plants are then investigated in detail, beginning with nutrition, partly because of its fundamental nature, and partly because most of its phases can be studied well in the winter, leaving the spring for the study of growth and irritability, which need the brighter and warmer season. Topics which cannot be practically studied, but are nevertheless important, are introduced in their proper places in order to keep correct proportion, as already explained; paragraphs and connecting sentences are introduced here and there to show the relation and connection of topics and to help to bind the whole into one symmetrical system. It may seem at times that too great stress is laid upon comparatively unimportant topics, but for this there is always a reason. Thus, a good deal of attention is given to the responses of protoplasmic movement to variations of temperature, although that subject is in itself not nearly so important as many others more briefly treated. The reason for the apparent emphasis is that this particular topic, coming very early in the course, affords a remarkably good opportunity to introduce students to exact quantitative methods, and their expression by tabulation and construction of curves; and to the methods of elimination of individual errors. The theory of every experiment is given and the reason for each step; and, for completeness, references are added to other ways of attacking the same or related problems. Here and there synoptical essays are called for, on the scope and value of which some comments will be found in the next chapter. Supplementary or correlated topics, not belonging in the main framework of the course, are mentioned at the ends of the sections with which they are connected; these topics may well be discussed by the teacher in the lectures. All of the topics, for reasons already given, i.e., to bring them into the form of investigations, are presented as problems introduced by questions. The form of these questions, and their number and sequence, are of great importance, and have been given much study. Through them the students' energy may be concentrated, their attention directed in the most profitable lines, and their mode of attack on the problems made inductive; while through them, too, much both of stimulus and suggestion may be conveyed.

The course as here outlined is completed by my own students in one college year, working each week about eight hours in the laboratory, with one lecture and one hour devoted to criticism of experiments and comparison of results.

The publication of the English edition of Part I of Pfeffer's full and authoritative "Handbook of Physiology," with its many citations of literature, and the probable early appearance of Part II, render unnecessary any special references to literature. It is assumed that his work, together with such standard books as are mentioned at the end of the next chapter, are in the laboratory and constantly used. Special papers, accordingly, are only cited where their reading is particularly profitable, as, for example, where they contain remarkably good summaries in English by experts in the particular topics, or, in the case of original contributions, where they are particularly illuminating to the topic under discussion.

2. TEACHING AND LEARNING.

It is altogether probable that wherever such a course as is outlined in this book is taken up, it will be under direction of one who is both a trained botanist and a skilled teacher, and that any students electing it will already have acquired some skill in scientific ways of working and thinking. Hence it may seem superfluous to volunteer advice upon teaching or learning physiology. Yet discussion and comparison are essential to progress, and therefore I shall give in brief synopsis some of what seem to me the characteristics of good teaching and good learning in physiology, adding thereto some suggestions, based upon personal experience, as to profitable procedure in the physiological laboratory.

The general principles of good scientific teaching apply in full force to a practicum in physiology. The true teacher, for his part, is a liberal but firm leader, a genial though uncompromising critic, a sympathetic and helpful friend. He studies well the mental character of each student, and quietly treats each in the way best for itself. He teaches largely through example, aims for optimum rather than maximum results, and seeks to inspire his students to do rather than to know. He utilizes the good and pleasurable instincts in his students, their curiosity, their pleasure in competition, their artistic sense, their ambition. At each new step in their work he recalls to them what they already know, and from this vantage-point in the known he leads their sorties into the unknown. He tries to create a demand for truth before he provides the supply. He habitually illustrates proper inductive procedure and the scientific use of deduction; makes clear the true function of observation, hypothesis, experiment; and emphasizes training in the logic of evidence,—the power to distinguish between the practically proven, the degrees of probability, and the merely possible. He does not shrink from discussion with his students, nor refuse to learn from them. Finally, he is not discouraged by the inevitable discrepancy between his ideals and his results, but, remembering that averages and not extremes count in the long run, he presses cheerfully on, profiting by experience and building for the future.

The successful student in this course recognizes that his teacher's duty is simply to provide opportunities and advice, while his own is to take advantage of them. He studies with care the methods and results of the masters in his subject, tries to see from their point of view, and seeks to acquire their open, judicial, evidential habit of mind, which alone can lead to scientific success. He acquires deliberation and self-reliance, a desire to go always to original sources of information, and a preference for knowledge acquired through his own efforts to that derived from any other source. He comes to admire results founded upon exact evidence and logical reasoning, and to distrust and dislike conclusions based upon insufficient, badly grounded, or emotional data. He believes only when convinced, does his own work, asks for aid only when it is needed, and profits both by his own mistakes and by the successes of others. As to the more practical aspects of his laboratory work, he is exact and neat in everything; he not only keeps his own place and property in good order, but takes a corporate pride in the appearance and condition of the laboratory as a whole; and he works in physical comfort and with a feeling and an air of academic calm and leisure.

For the profitable pursuit of a course in physiology, some training in chemistry and physics is essential, a knowledge oı German is extremely desirable, and some knowledge of zoology and meteorology very advantageous.

We may next briefly consider actual laboratory procedure. It is essential that laboratory guides or outlines, to give direction and definition to the work, be placed before the students, either as offered in this book or in some related form. The materials for use in experiments are in some known place, and a model or photograph of the more complex appliances is placed at their disposal. They then make their own attempts at the solution of the problems, aided only by the outlines. Each student has of course his own place in the greenhouse and in the laboratory, all of which he is expected to keep in the best of condition. All experiments should be set up in a mechanically neat and exact, one may even say an artistic, spirit. It is very profitable, even essential, to devote an hour a week, when all students are present, to criticism of experiments and comparison of results.

To secure exactness, completeness, and permanency in the students' work, very careful records both of procedure and results should be made. For this purpose I have found most advantageous a good laboratory note-book, stamped with the name of college and course, specially made with strong linen binding, 8½ by 7 inches, with 200 numbered pages of the best paper, ruled on the right-hand page for notes and unruled on the left for drawings.* It may seem that so elaborate a book is needless and that a cheap book, purchasable at any bookstore, is as good. But I have observed that the pride and care

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* Supplied by the Cambridge Botanical Supply Co., Cambridge, Mass.

taken by the students in the best obtainable book is much greater than with a cheaper one, not to mention the advantage of its much greater chance of preservation. In these books the students record results, using all care and taking an artistic pride in their completeness and neatness. Each record should be made complete in itself and a model of scientific exposition. It should include:

(1) A statement of the exact object of the experiment.

(2) A description of the practice and theory of the method used, with illustrations of the appliances. The latter may best be annotated blue-prints made by the students themselves from a negative of one of the experiments actually set up. It is not profitable to spend time upon drawing apparatus, though much drawing of diagrams, etc., must be done. In the latter free use of colors should be made, for which colored pencils are sufficient.

(3) The exact results obtained, expressed in tables of figures and in curves (or polygons). Whenever possible it is well to construct a curve averaged from the total results of the class, which for comparison is to be plotted on the same sheet with the individual's curve. Indeed this method offers the simplest means for obtaining an approximate idea (the more accurate the larger the number of students whose records are combined in the average) of the probable error in the individual's results, whether this be due to the "personal equation" of the observer, individual variation on the part of the plant, or uncontrollable variations in external conditions.

(4) The conclusions as to the result of the experiment, together with remarks as to its general bearings.

In their proper places in the sequence of topics should come the accounts of the subjects studied non-practically and the essays. These essays are of particular value. They are by no means designed to include details already embodied in the laboratory books, but are studies in proportion, generalization, condensation, and expression. They demand in their writing a correctly proportioned and logically complete knowledge of the subject covered by them, and enable the teacher to judge whether the student has acquired it. To prevent diffuseness it is needful to restrict their length, and it is well to have them preceded by a tabular synopsis of contents. So well does the laboratory work and book keep the teacher informed upon the student's progress, and so well do the essays accomplish the purposes of review, that examinations are quite unnecessary, and the time saved from them and their preparation may be turned into the course work.

The students should be required to complete particular problems, and to make full records of them, before they submit results for the teacher's inspection. They naturally tend at first to ask whether each step is correct before they proceed to the next; but, if this be allowed, soon the students are doing the mechanical and the teacher the mental work. Pride in the neatness of their books, too, leads the students to dislike to mar them by corrections. But the books are simply a record of the owner's work, and it is essential to the formation of habits of self-reliance that students should carry work through to completion the best they can for themselves. When thus completed the records should be critically examined by the teacher in consultation with the student, and corrections made or approval expressed. In my own experience I have found it profitable to use a short oblique mark at the outer lower corner of pages examined, making it a cross for pages that are satisfactory; and the students are held responsible for having the corrections examined and all pages crossed. But all such devices, and labor-saving methods generally, must not be allowed to become dominant, and give the course an aspect or a spirit of formalism; they must be rigidly subordinated to the liberal, scientific, optimistic spirit in which such a course should be carried on.

A course in physiology hinges largely upon experiment, and a clear idea of its real nature and true function is therefore essential. A really good experimenter is born, not made, for there is a sort of experimental instinct, which is complex and includes inquisitiveness, faith in one's own powers, pleasure and natural skill in mechanical manipulation, and ready perception of the value of evidence. All of these may, however, to some extent be cultivated. An experiment in its essence is a question asked of nature, and should always be the direct definite question of a thoughtful seeker after knowledge. It should call for as simple an answer as possible. It properly follows upon careful observation, and usually is a testing of hypotheses suggested by reasoning thereon. Most commonly an experiment is undertaken to find out the relation existing between the processes of the plant and some particular external condition; and practically the first and most natural step is to observe the effect upon the plant when that condition is removed or neutralized. The ideal experiments are those in which only the single condition is altered; but, partly on account of the closeness with which different conditions are yoked together, and partly because of the relative crudeness of even our finest methods of experimenting, this is very rarely possible. Hence in order to make sure that the result obtained is really connected with the condition changed, and not with some secondary influence introduced by the manipulation in the experiment, it is usually necessary to try at the same time a parallel experiment in which a similar plant is placed under precisely the same conditions as the first plant except that the given single condition is not changed. Here, in both experiments, all the secondary conditions are the same, the difference is only in the given primary condition; and hence it is a fair inference that an observed effect is connected with the change in the primary condition. Such an experiment is called a *control*, and an impulse to control experimenting is an essential part of the experimental habit. It is in order to minimize the danger of disturbance through the introduction of secondary conditions that mechanical neatness and exactness are so essential in experimenting.

In recording results, in addition to careful tabulation, these should, whenever possible, be plotted in curves or polygons. Such curves not only have the great merit of expressing results clearly to the eye at a glance, but they bring out facts and relations altogether unsuspected from even the minutest inspection of tables of figures. Moreover their effect upon the students is extremely good; they encourage greater accuracy, more critical study of topics, a better understanding of the significance of results, and a greater general interest in the work. The curves are, of course, especially valuable where more than two sets of conditions are to be compared, as, for instance, where one wishes to compare the rate of transpiration with the variations in temperature and in moisture, but they are valuable in all cases where it is desired to express quantitative relationships. Such curves are plotted on co-ordinate paper ruled in faint colored cross-lines; the abscissæ or horizontal lines are used for the degrees of the external condition which alters steadily (as, for instance, temperature), and the ordinates or vertical lines express the degrees of the effect upon the plant; the joining of the tops of the ordinates gives the curve or, more properly, polygon showing the relation between the conditions expressed by ordinates and abscissæ. A particularly favorable opportunity for introducing the students to these methods is offered by the study of the relations of protoplasmic movement to temperature (page 54). It is well for students not only to construct their own curves in this and other cases, but also to unite and construct an average or class curve which each student plots with his own for comparison. To allow of this averaging, the different records must, of course, be made upon the same basis. This class curve has also the advantage already mentioned, that it offers a convenient, even though not very exact, method of allowing the student to ascertain approximately the probable error in his own individual results. As an example of class and individual curves there is here given (Fig. 1) the curve obtained by averaging the results of nine students for the relations of protoplasmic movement to temperature in a tip cell of Nitella, and with it is given an individual record taken at random.

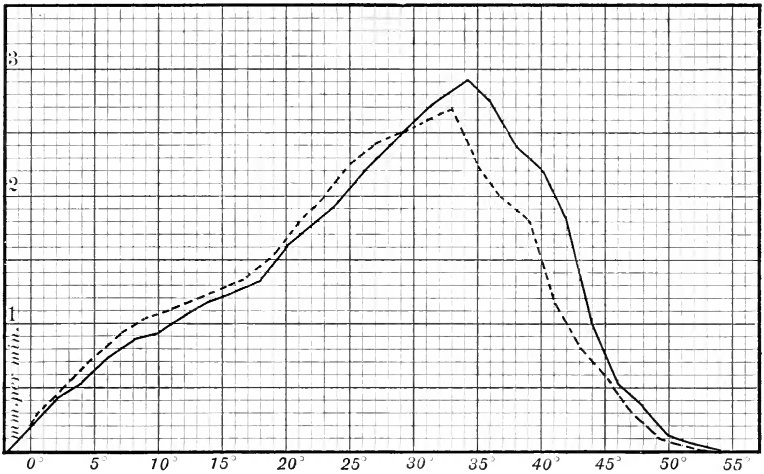

Fig. 1.—Curve (Polygon) of Protoplasmic Movement in Nitella under Varying Temperatures.

The continuous line is an average of the results of nine students; the broken line is the result of one student.

Of very great value, too, are the generalized diagrams often called for in this course. Nothing can surpass them for securing clearness of vizualization.

In a course such as this, which brings the student so often to the borders of philosophy, some attention must be given to theories and theorizing, and even to speculation. There are many, however, who will not agree with the wisdom of laying so much stress upon these as is done in the following pages. Certainly their profitable educational use depends upon the temperament of the teacher. Any teacher who does not naturally take a deep interest in such matters, or who does not feel confidence in his ability to use this method without losing control of it, had better not attempt to use it extensively, if at all. Theorizing has this justification, that the current theories to explain important phenomena have much value as knowledge to persons of culture, and they give a life and significance to facts otherwise of little meaning. They stimulate interest and mental activity, and are actually an invaluable tool of scientific research. But no student is prepared to understand the place and bearing of the leading theories unless he has tried to develop for himself some interpretation for the facts those theories explain. Students receive with but a languid interest such a theory as that of the micellar basis of membranes, or that which explains osmotic pressure, when these are formally presented to them; but they receive these theories with an eager and intent interest when offered after they have tried themselves to devise a theory to explain the facts they have observed in their studies upon osmosis. All such theorizing, however, must be kept in rigid control, subordinated to facts, a means to an end, never an end in itself. The tentative and insufficient nature of even the most widely accepted theories should be illustrated by subjecting them to rigid criticism. It must be made clear that theories are mostly attempts to explain in a subjective form phenomena which may not be subjectively comprehensible at all. In the exercises involving theorizing in this course, it is not expected that students will be able to solve the many theoretical problems proposed to them; but it is intended to impress upon them the fact that such problems exist, and to lead them to exercise their minds and prepare their attention through attempts to solve them.

Lectures are of great value to correlate and extend topics and to help to weld them into one harmonious system. They should be studies in induction, proportion, apt illustration,* suggestion, and stimulation of interest. Disconnected from laboratory work they have little value, but closely following

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* Wall diagrams for illustration of lectures, etc., have their value. Two sets have been published for physiological use—an earlier, 60 in number, each 69 × 85 cm., by Frank and Tschirch (published by Paul Parey, Berlin, and costing 180 marks), and a later, 15 in number and somewhat larger, by Errera and Laurent (published by E. Lamertin, Brussels, and costing 50 francs). They may be imported through any dealer in foreign books.

the latter, as they should, they have a firm foundation to build upon. Every topic personally studied in the laboratory by a student becomes a centre of illumination for a large circle of theoretical study, which, without the practical work, is misty and well-nigh meaningless. Such topics are like determinations of latitude and longitude in the construction of a map; they give an absolute location of a number of the most important points, allowing the intermediate topography to be sketched in without sensible error. It is for this reason that topics calling for book and lecture study here and there in the course are entirely profitable.

To learn to use literature properly is an essential part of a scientific education. A habit should be formed of consulting the original papers whenever possible, and of comparing the work of different authorities upon the same subject. The great accumulation of literature makes it necessary also not only that the student shall be able to read absorptively and critically, but also that he acquire the power of extracting the substance of a work by skimming its pages or from inspection of its tables of contents, figures, or summaries. In such a course as this, however, supposed to be taken by undergraduate seniors, it is obviously impossible to carry the consultation of the original literature very far, but enough of it should be done to emphasize its value. Original papers can at least often be brought into the laboratory and looked over, even if not read. Happily, however, for such a course as this, the literature is admirably summarized in Pfeffer's "Handbook of Physiology," of which the first half has appeared and has been translated into English. It is assumed throughout this course that the student carefully reads this indispensable book. He should also consult Noll's "Physiology" in the Bonn Text-book, Vines' "Text-book" and his older "Lectures," and Sachs' "Lectures," though the two latter must be used with some caution as they are not up to the times in their facts. Sachs should be read as a model of scientific exposition, expressed in an attractive style. Very suggestive and valuable for its breadth and point of view is

Verworn's "General Physiology," though most of it is not botanical. Goodale's "Physiology," though much behind the present state of knowledge, is a useful synopsis of some topics, and Sorauer's "Popular Treatise" has considerable value for the economic aspects of the subject. Schimper's "Pflanzengeographie" is indispensable for the ecological phases of physiology. A recent and readable summary of the subject is Green's "Introduction to Vegetable Physiology." Again, it is very profitable in connection with experimentation to read another work upon the experimental phases of the subject, and for this nothing is better than Detmer's "Practicum," translated into English by Moor under the title "Practical Plant Physiology." The student should read this book as regularly as Pfeffer's, in both cases after the laboratory work on the particular topics. It is well also to consult Darwin and Acton's "Practical Physiology" and MacDougal's "Experimental Plant Physiology." MacDougal's "Nature and Work of Plants" has many suggestions for elementary physiological study.

Following is a bibliography of the works indispensable to the working laboratory.

Darwin and Acton. Practical Physiology of Plants. Second ed. Cambridge, 1897. 4s. 6d.

Detmer, W. Practical Plant Physiology. Translated by Moor. New York, The Macmillan Co., 1898. $3.00.

Goodale, G. L. Physiological Botany. New York, American Book Company, 1885. $2.00.

Green, J. Reynolds. An Introduction to Vegetable Physiology. London, Churchill, 1900. 10s. 6d.

MacDougal, D. T. Experimental Plant Physiology. New York, Henry Holt & Co., 1895. $1.00.

——— The Nature and Work of Plants. New York, The Macmillan Co., 1900. 80 cents.

Noll, F. In Strasburger, Noll, Schenk and Schimper, A Text-book of Botany. Translated by Porter. New York, The Macmillan Co., 1897. $4.50.

Pfeffer, W. The Physiology of Plants. Translated by Ewart. Oxford, the Clarendon Press. Volume I. 1900. 28s.

Sachs, J. Lectures on the Physiology of Plants. Translated by Ward. Oxford, Clarendon Press, 1887. (Out of print.)

Schimper, A. F. W. Pflanzengeographie auf physiologischer Grundlage. Jena, Gustav Fischer, 1899. 27 marks, unbound.

Sorauer, P. A Popular Treatise on the Physiology of Plants. Translated by Weiss. London and New York, Longmans, Green & Co., 1895. $3.00.

Verworn, M. General Physiology. Translated by Lee. New York, The Macmillan Co., 1899. $4.

Vines, S. H. An Elementary Text-book of Botany. New York, The Macmillan Co., 1898. $2.35.

——— Lectures on the Physiology of Plants. Cambridge, University Press, 1886. 21s.

(Arthur's "Laboratory Exercises" and MacDougal's "Plant Physiology," cited in the Preface, may be added, but are not regularly listed as published works.)

3. GREENHOUSE AND LABORATORY.

It goes without saying that a Greenhouse and a Laboratory are indispensable for a course in Physiology. The more excellently built and equipped these are, the better; but good work depends not upon them and their furnishings so much as upon the spirit of teacher and students.

The greenhouse and laboratory should adjoin one another, and the ideal place for them, for convenience of heating, care, supply of materials, etc., is in connection with a range of scientific greenhouses in a Botanic Garden. But of course a small greenhouse may be built off a laboratory in an ordinary building, the more especially on a top floor where some angle or flat roof may offer a convenient position. The great requisite in such a house, one indispensable where most of the work is done in winter (as it generally is in this country), is that it shall have full exposure to light without being shaded by neighboring structures for any appreciable part of the day. It is for this reason that Wardian cases built into windows, or larger structures of the same character, while ample for purposes of elementary experimentation in general courses, is insufficient for such a course as this, though a part, perhaps one half, of the course could thus be carried on. A greenhouse built off the laboratory is likely to offer difficulties about heating unless a steam or hot-water system is available, but there are systems of heating by stoves which any builder of greenhouses can give advice about. As to cost, it is impossible to give any exact estimate, since this depends upon size, permanency, and many other considerations. Small houses, such as are furnished for amateur horticulturists, may be bought for from three hundred dollars upwards; while such a greenhouse as is described later in this chapter, built for the utmost convenience, completeness, and permanency, if heat is available from a neighboring building will cost at least fifteen hundred dollars, while the laboratory described will cost about twelve hundred dollars complete. If nothing else is available, it may be possible to hire a portion of a commercial greenhouse in the vicinity. But economical, accurate, profitable physiological work cannot be done without a good greenhouse.

Such advice as I can offer upon the building and equipment of a physiological greenhouse and laboratory may be given in the form of a description of the plans for proposed new houses for Smith College, which embody the best I have been able to develop from my own experience. Sketches of the plans will be found in Figs. 2 to 6. The shape of the houses there given is necessitated by the nature of the ground and the position in which they must adjoin the present range of greenhouses; probably were this condition not present it would be better to make them both more nearly square.

These houses are planned for a class of twelve students, together with three or four students working upon special topics. The greenhouse (Fig. 2) is 32 by 18 feet inside measurement, with brick walls a foot thick rising above the floor 3 feet 6 inches, above which is glass to a height of 6 feet, while the center of the roof rises to 12 feet. Ample ventilators are needful both on sides and roof. The floor is everywhere cemented except where the pits for the heating-pipes occur. These pipes must either be sunken under the floor and covered with iron gratings, or else suspended along the walls in a single vertical row; for if placed above the floor in the usual way they will render impossible the separate tables which are so essen-

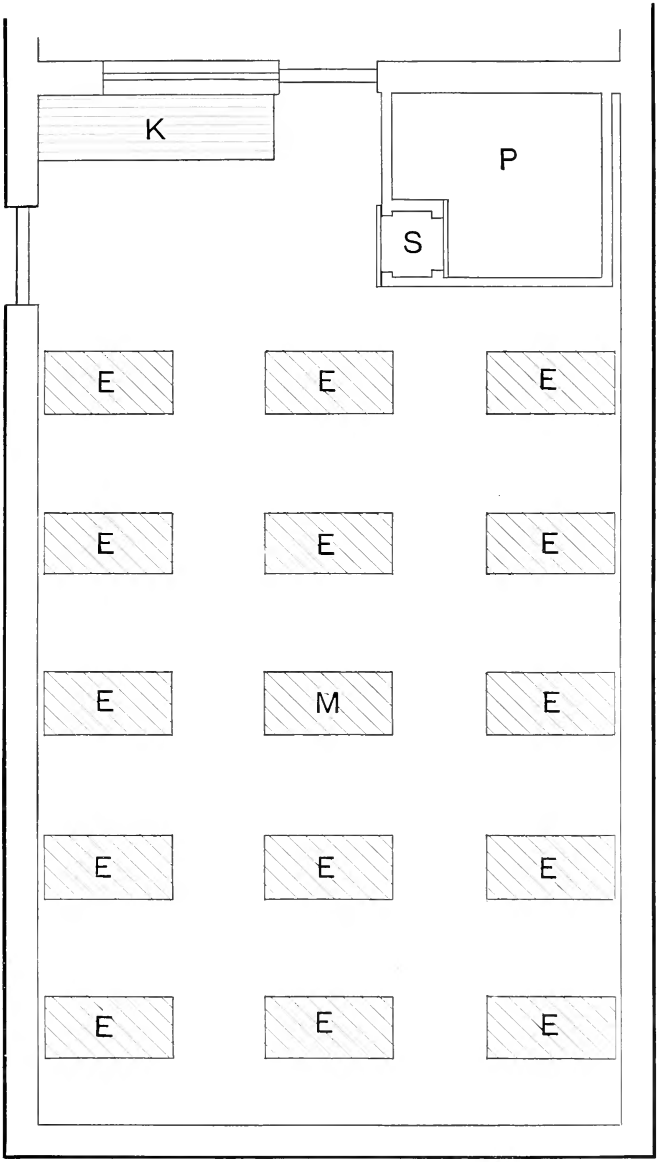

FIG. 2.—GROUND-PLAN OF EXPERIMENT GREENHOUSE.

E, experiment tables; *M*, table for meteorological instruments; *P*, physiological dark-room; *S*, entrance to dark-room; *K*, sink. Scale, 1 in. = 6 feet.

tial to a good experiment house. The pipes must be abundant enough to allow a high temperature, but should be controllable by valves so that it may be lowered to any desired degree. The fifteen separate tables, each 4 feet by 2, and 3 feet 6 inches high (thus high for greater convenience of working standing), are iron-framed, and braced diagonally so as to be immovable when touched (Fig. 3), and have tops of two pieces of smooth slate, which are supported on levelling-screws at the four corners so they may be set level at will, or else are set permanently level in cement. The middle one is covered with a shelter of the meteorological sort for thermographs, hygrographs, and other needful instruments. Very essential is the large porcelain-lined sink with five taps, to three of which are attached respectively a Chapman exhaust, a Boltwood blast, and a small water-motor. A constant-temperature chamber is needed for some purposes, and this can well be obtained by use of such a Wardian case, heated by gas, controlled by a thermo-regulator, as I have elsewhere described.* It should be set in one

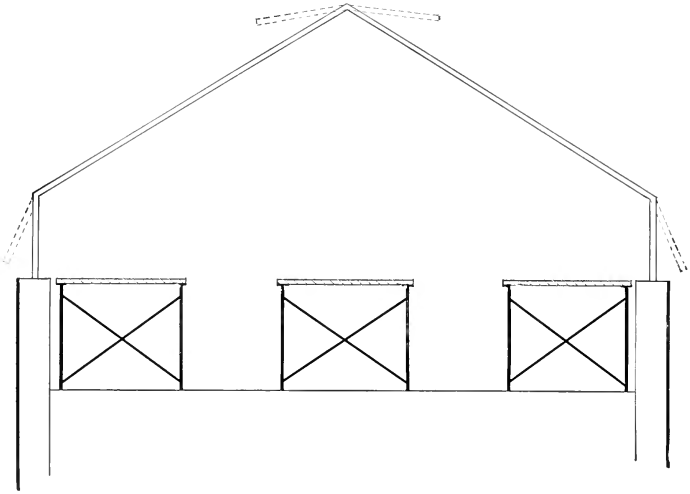

FIG. 3.—SECTION THROUGH EXPERIMENT HOUSE. (Compare with Fig. 2.) Scale as in Fig. 2.

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* "The Teaching Botanist," page 83.

of the outer corners (lower one in Fig. 2) and have a special pipe to carry the combustion gases out of doors. It can be darkened by a dark hood of cloth, and can be kept running to within two or three degrees of a given point. Of course for some investigation purposes an underground chamber is better, but it seems needless for such work as is here supposed to be carried on.*

Of the very greatest importance in this course, and, indeed, essential for most physiological work, is a well-ventilated dark-room. This is to be constructed as shown in Figs. 2 and 4. The walls are to be of one thickness of brick on all sides except the laboratory partition, which is to form the fourth side. A space separates it from the outer greenhouse wall in order to help keep its temperature at that of the greenhouse. It must have two doors, made light-tight by rubber flaps, with a space between them such that a person can enter and close the outer door before opening the inner; by this arrangement light may never enter. The construction of the roof is important (see Fig. 4). The glass of the greenhouse above it is to be painted

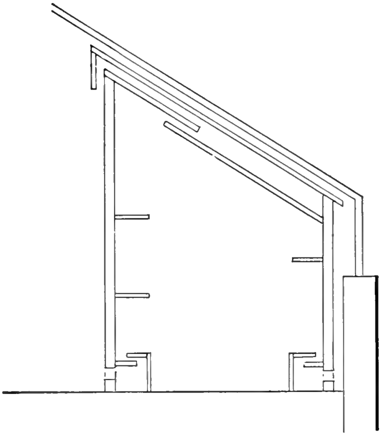

FIG. 4.—SECTION THROUGH PHYSIOLOGICAL DARK-ROOM.
Scale as in Fig. 2.

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* Temperature-chambers (thermostats) of all sizes up to rooms, and claimed to be of great exactness in operation, are a specialty of the firm of Dr. Hermann Rohrbeck, Karlstr. 20a, Berlin, N. W.

opaque white to reflect as much light and heat as possible so the dark-room will not become too much heated. The outer roof of the dark-room, placed just below the greenhouse roof, is of matched boards painted white above and black below and with a special projection in front as shown in Fig. 4. Beneath this comes a double roof all painted black, arranged as shown in the figure, to allow the escape of air, forming a part of a ventilation system. Entrance for the air is arranged thus: in the row of bricks just above the bottom row on the three thin walls, each alternate brick is to be omitted, and a wooden box, painted black, in the form shown in section in Fig. 4, is to be built all around the three sides (except of course where the doors are). This arrangement will admit air freely but no light, and the air will escape without admitting light through the double roof above. A small dark-room, or rather box, on this principle has worked well through two years' use. The brick outer walls may be painted white; and shelves are to be placed at convenient heights on the inner walls. The floor is of cement. The heating-pipes do not run into the room, as the air-space extending practically all around will make it hold approximately the temperature of the greenhouse.

The shading of every greenhouse from the too intense sun of some days is important. This can be effected by the usual whitening of the glass in the spring, but for physiological purposes it is much better to have a system by which the shading is not only removable at will, but is alterable in different degrees. This can be effected, though with some trouble, by having lengths of cheese-cloth which can be pinned to the beams of the greenhouse roof by spring clothes-pins. What appears to be a better system is to be tried in our new greenhouse. Stout galvanized-wire frames, 8 feet long and 6 inches wide, are to be covered with thick cheese-cloth and hinged by one edge to the greenhouse roof, stretching across between the beams. A cord is to connect the middles of the lower edges, and by drawing on this cord the frames can be set parallel with the sun's rays, when they will give practically no shade at all,

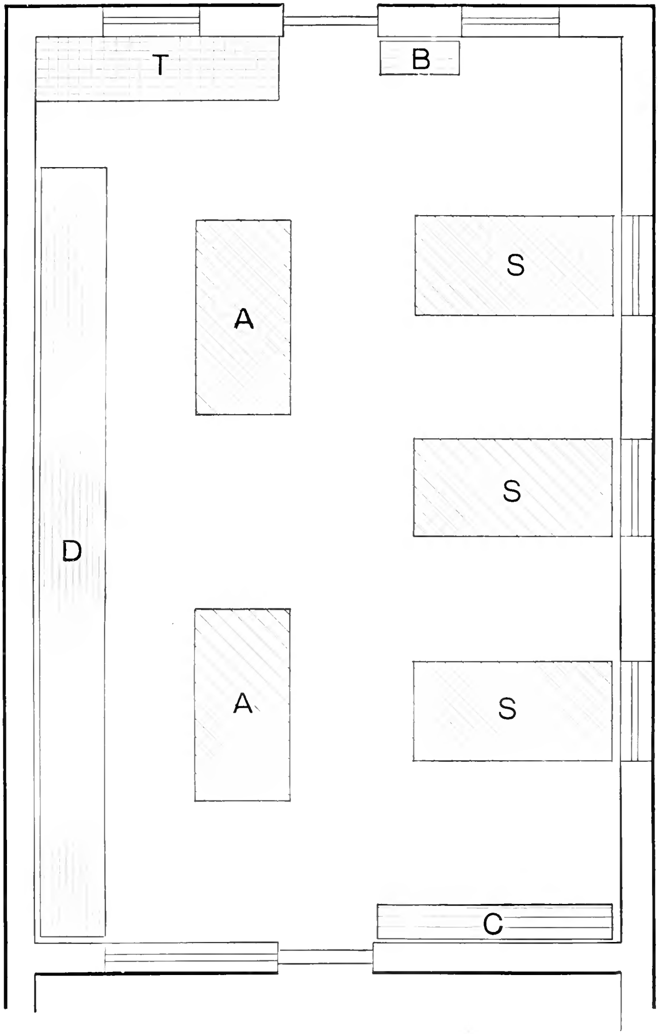

FIG. 5.—GROUND-PLAN OF PHYSIOLOGICAL LABORATORY.
A, apparatus-tables; *B*, books; *C*, chemicals; *D*, apparatus-cases and drawers; *T*, gas and-tool table. Scale as in Fig. 2.

or they may be set at right angles, when they will shade the house completely, and all intermediate degrees may be obtained. Of course the intermediate degrees are given by bars of full light and shade, but the experience of gardeners shows that this slat-shading is perfectly efficient.*

Passing next to the Laboratory we find its construction much simpler. It will be built of brick, some 28 by 18 feet inside measure (Fig. 5), and at least 9 feet to the ceiling. Tables for twelve students are needed, especially for microscopic and other fine work, and these may well be of the usual laboratory form and size, i.e., as shown in section in Fig. 6, some 6 feet long (for four students), 3 feet wide, and 2 feet 6 inches high, with a vertical row of drawers on the middle of each side. Tables for the assembling of apparatus are needed, which, as they are used standing, may best be 3 feet high; and they may have lockers beneath for storage (Fig. 6). Very

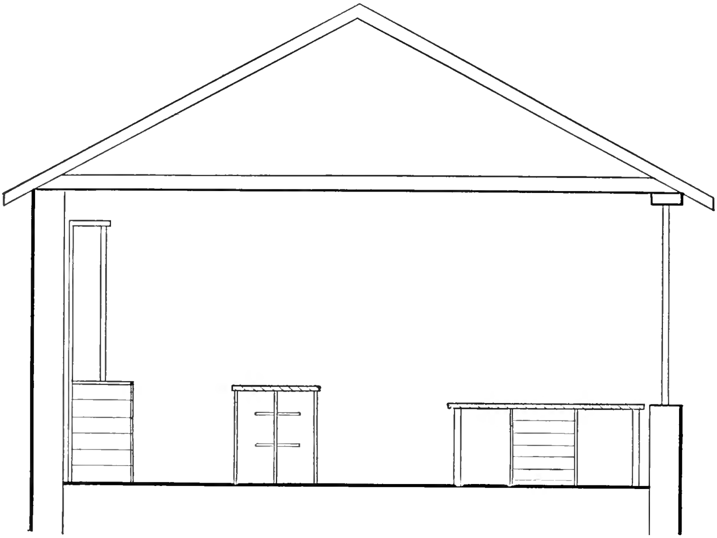

FIG. 6.—CROSS-SECTION THROUGH PHYSIOLOGICAL LABORATORY. (Compare with Fig. 5.) Scale as in Fig. 2.

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* There are references to descriptions of experiment houses in Detmer, page 8, foot-note 1, but these I have not myself read.

important is a tool-and-gas table equipped with bunsen and other burners, and the ordinary tools as described in the next chapter. This should be 3 feet high, and the space beneath it used one half for drawers and the other for cupboards. So much apparatus is needed in this course that ample storage room must be provided, and hence the long case shown on the plan, with numerous drawers beneath, and glass-fronted cases above, will be none too large. Separate cases should be provided, too, for books and for chemicals; and radiators, or, better, lines of heating-pipe along the walls beneath the windows, are necessary. A case for the balances may well stand on one of the apparatus tables. The windows should be broad and tall, and should be opposite the student's tables and the tool-table, while one half of the partition between laboratory and greenhouse may best be of glass. Such seems to me a profitable general arrangement, though a far simpler one allows of extremely good work.

4. APPARATUS AND MATERIALS.

A list of the appliances and materials needful for a practicum in physiology will now be given. It is not always easy to determine whether certain given pieces shall be included or excluded, since the scale of equipment is a sliding one which can be considerably expanded or contracted according to circumstances; but as a standard I shall take the equipment actually used by my own class of twelve students in the present (1899–1900) year. This may be taken to represent an optimum of equipment for such a course, but some of the articles could be omitted without serious detriment to the work. Although at first glance the list may appear formidable in length, it is really hardly longer than would be a list of the equipment of a good anatomical course with its microscopes, microtomes, paraffine baths, etc. Moreover, it is not needful to procure all of the appliances at once, but they may be added a few at a time from year to year; besides, most of the appliances and materials needed are of the simpler and least expensive sort and may be used for many years. For the most part they may be bought from the laboratory fees usually charged in colleges. Even the cost of the full equipment does not surpass, if it equals, the cost of equipment of a good anatomical laboratory.

While only the actually necessary appliances are here to be mentioned, the teacher will of course accumulate other simple appliances and the commoner chemicals as he continues to improve his experiments and to invent new ones. It is desirable, too, as already pointed out, to add some of the more exact and complicated appliances for demonstration purposes and occasional exacter measurements, but these are not at all necessary.

Prices of most of the articles needed, when not here given, may be found in the catalogues of any of the large dealers in chemical supplies. The larger articles may be imported through them duty-free for educational institutions. The apparatus used in all of the exercises in Detmer's "Physiology" is supplied by Desaga, Heidelberg, while numerous very useful pieces are sold by Professor J. C. Arthur, Purdue University, Lafayette, Ind., from whom descriptive price-lists may be obtained. Most of Professor Arthur's apparatus is described in the *Botanical Gazette*, **22**, 463–472. All apparatus and other supplies are furnished or imported by the Cambridge Botanical Supply Company, Cambridge, Mass.

A. APPARATUS USED IN COMMON BY ALL OF THE STUDENTS.

A gas-table, 3 feet 6 inches high, with bunsen burners (about one to each two students); also a fish-tail burner, a water-bath with support, extra supports for beakers with wire netting, a small blowpipe, and a soldering outfit. A foot-bellows with a blast bunsen burner is useful but not needful.

A tool-table with the ordinary tools; also fine and coarse wire tweezers of the cutting kind; glass-cutter; 2 or 3 each of triangular, flat, and round files of different sizes; a vice. A small chest of thoroughly good tools, attachable as a closet to the wall, is very useful.

Chapman air-pump, attachable to a water-tap (costs about $3.00). It should be connected with a mercury manometer-tube to make the exhaust visible. A Boltwood water-blast is often useful.

Balances. For heavier work the Springer torsion-balance, costing about $14.00, is excellent; for transpiration experiments a delicate spring-balance is most convenient, for which the Mail and Express Balance (Pelouze Scale Co., Chicago, $5.00) is excellent. For a very accurate balance carrying heavy weights, the balance 3423 of Gerhardt, 115 marks, is to be recommended (see also page 79). For weighing chemicals the Troemer balance, costing about $7.00, is ample for most purposes and very convenient.

Metronome. Costs about $4.00.

Thermograph. Costs about $35.00, made by Richard Frères of Paris.

Hygrograph, or registering hygrometer. Costs about $35.00, made by Richard Frères of Paris.

Some form of sunshine-recorder is useful but not indispensable.

Photographic outfit. The camera should have a bellows long enough to allow the photographing of small objects at least their full size, and should take at least 5 × 8 plates.

Spectroscope. May usually be borrowed from the department of physics. A microspectroscope may be used, but the Kirchoff and Bunsen, costing about $30.00, is excellent and ample for this work; or the Hoffman form, made by Max Kohl at 260 marks, is said to be excellent. It must have a comparison prism (see Fig. 17).

Polariscope. Not much used, and not indispensable: used on the microscope only. The Zeiss polarizer and analyzer, listed at 39 marks, is ample.

A simple steam sterilizer. The Arnold form is not expensive and extremely good; one of the larger sizes is needed.

Measuring-glasses (graduates), cylindrical, preferably of 10, 100, and 500 cc.

Four funnels of different sizes, about 20, 13, 8, and 5 cm.

Receptacles for, respectively, molasses, nutrient solution, lime water, distilled water, alcohol. Aspirator bottles with ground-glass stop-cock outlets at bottom are very good; for the two former liquids they should be of a half-gallon capacity, for the three latter of a gallon capacity. The mercury bottle may best be a "separating-funnel" which should be placed over a tight wooden box to catch leakage (see Fig. 7).

Three dry-battery cells, with tray or board to hold them, with a simple circuit-closer.

Oil or whetstone; an emery wheel attached to a water-motor is very useful.

Simple still for distilling water.

Carbon dioxide generator, of usual simplest chemical form (Fig. 7).

Scissors and shears; scalpels.

Rubber stamp, marking small squares, and ink-pad (see page 106).

Bicycle-pump (preferably a foot-pump, costing 50 cents).

Spirit-level, 12 to 15 cm. long. Very valuable for some purposes, such as truing the cylinders of recording mechanisms (page 105), is a piece of slate 2 cm. thick and 30 cm. square, set on three levelling-screws.

Supply of porous flower-pots of different sizes.

Supply of glass tubing of the various smaller sizes, principally 1 mm. thick and 3, 5, and 7 mm. bore; also some capillary tubing.

Supply of rubber tubing of the different smaller sizes, principally 4, 8, 12, 16 mm. internal diameter, of soft black rubber, and some of the thick cloth-wrapped kind used for gas conduction, also one meter of the smallest obtainable.

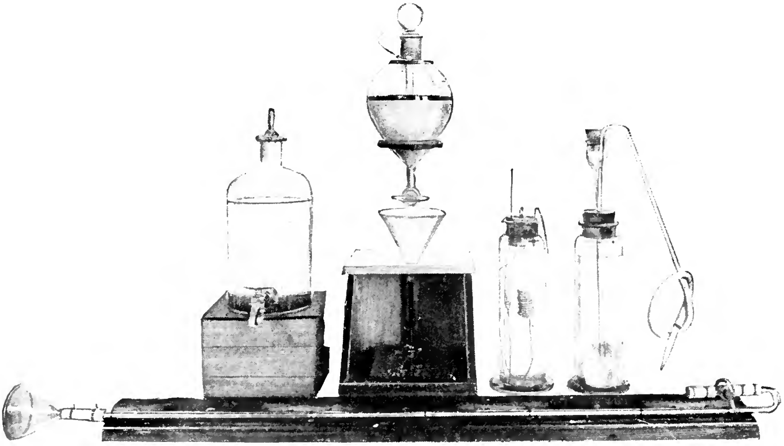

FIG. 7.—RECEPTACLES FOR CHEMICALS, ETC.

In the center, mercury reservoir; below, pressure manometer; on the left, aspirator bottle; on the right, phosphorus-carriers and carbon dioxide generator. All $\frac{1}{8}$ true size.

Supply of rubber stoppers; most useful sizes are 20, 24, 28, 32 mm. diameter at large end; also some with two holes (which can be stopped with sealed glass tubing if not needed).

Supply of good quality ordinary corks, different sizes.

Set of 10 cork borers, with sharpener, and a cork-presser.

Spools of copper wire of sizes 16 and 20, kept hanging over the tool-table. Also a spool of the finest wire; also some No. 20 elastic iron or brass wire.

Electrician's tape or tire-tape, large roll.

Cross-section paper; that ruled every 2 mm., with cm. lines heavier, is best.

Black paper in large sheets for dark hoods etc. An ideal paper for this would be black for the inside and white, to reflect heat, on the outside. Particularly good for hoods, etc., is a roll of the felt paper, 3 feet wide, used as drying paper in herbaria.

Roll of cheese-cloth.

Filter-paper.

Glue, mucilage, gummed labels, pins (very long and slender), tacks, rubber bands, brass paper-fasteners.

Strongest linen thread, "carpet thread" (or No. 20 or 30); twine.

Fine silk thread.

Dentist's rubber sheeting, medium thickness (can be dispensed with; see page 79).

General glassware.

Parchment paper and tubing (smallest size).

Higgins' waterproof India ink.

Bottle of chronographic ink.

Black sealing-wax, soft.

Blue-print paper (obtained fresh from time to time) and printing-frames.

Tracing-linen.

Beeswax, hard paraffine, and vaseline.

Strips of soft flexible brass (such as "paper-fasteners" are made from).

Small can of good varnish; pot of dull black paint. Brushes should be kept in stoppered bottles.

Panes of good thin white glass of various sizes.

Plaster of Paris.

B. CHEMICALS USED IN COMMON BY ALL OF THE STUDENTS.

(Approximate amount used by twelve students in one year.)

Mercury, 15 kilos. (usable year after year).
Pyrogallic acid, 100 g.
Caustic potash, 1 kilo.
Alcohol, 5 liters.
Potassium ferrocyanide, 100 g.
Copper chloride, 50 g.
Molasses, 1 liter.
Coarse salt, 2 kilos.
Potassium iodide, 25 g.
Iodine solid, 25 g.
Olive oil, 2 small bottles.
Safranin, small bottle.
Soda lime, ½ kilo.
Phosphorus, 100 g.
Sugar, granulated, 1 kilo.
The aniline dye, "scarlet," 25 g.
Calcic oxide for lime-water.
Formaline, 200 cc.
Hydrochloric acid, 200 cc.

Small quantities of the following for making nutrient solutions:

Calcium nitrate.
Potassium chloride.
Magnesic sulphate.
Potassium phosphate.
(According to Detmer, 2.)

C. APPLIANCES NEEDED FOR EACH STUDENT.

(Those marked with a star need only be supplied one to each two students.)

Microscope, with forceps, two needles, camel's-hair brush, six slides, twelve cover-glasses, pipette, (microscopes may usually be borrowed from the anatomical laboratory, as they are needed at times only, not continuously).

* Clock-clinostat (see Fig. 32).

Eye-piece micrometer.

* Auxanometer. Dollar Waterbury clock, double wheel, and cylinder, as described on page 102.

* Temperature-stage, as described on page 55.

2 thistle-tubes, medium size.

2 burettes, 16 mm. diameter and 50 cc. capacity.

1 (better 2) iron supports (as in Fig. 10) with burette-holder and clamp.

1 diffusion-shell, Schleicher and Schuell, 16 mm. diameter (obtainable from Eimer & Amend, New York).

1 sheet best-quality glass, 30 cm. square, with corners removed or rounded, or round, 30 cm. diameter, and three wooden legs to support it, as shown by Fig. 29.

2 good centigrade chemical thermometers, graduated from 0° to above 100°.

2 plain calcium chloride tubes.

2 wide-mouthed bottles of about 500 cc. capacity, with corks to fit.

2 100-cc. plain funnels.

1 30-cm. graduated wooden scale.

3 largest size stout U-tubes, arms about 15 cm. long, with soft rubber stoppers to fit one arm, and slender bottles to hold them (see Fig. 21); or else, better, the three bulb tubes described on page 97.

2 concave watch-crystals and clamp (see page 82).

Small candle.

4 Zurich germinators (page 108) with pulp-plate and water-bottle, or else 8 small and 4 somewhat larger flower-pot saucers.

3 plain saucers.

3 plain tumblers.

* 2 crystallizing-dishes, 12 cm. diameter, and 5 cm. deep, with good corks that just fit them.

2 bell-jars, 15 by 30 cm. inside diameter, preferably with ground-glass stoppers and ground-glass edge, with ground-glass plate to cover bottom.

2 upright test-tubes or equivalent.

1 small test-tube.

1 battery-jar (see page 78).

A 3-inch slide with a slight smooth hollow.

A germination-box (see page 117).

1 large fibre saucer (see page 108), useful for many purposes.

Nest of ten beakers.

3 or 4 cheap wooden millimeter scales 30 cm. long (costing about 3 cents each from educational supply companies).

5 Soyka flasks for chlorophyll and color screens.

D. PLANTS.

Following are amongst the most useful plants for physiological study:

Sphagnum moss, obtainable in most bogs, and usually kept for use in greenhouses. It may be bought in bales (for about $1.50 per cubic meter) from dealers in seeds and greenhouse supplies. The dead moss obtained from below the surface of bogs is much better for germination experiments than the surface living layers.

Seeds of beans. Horse-beans (Vicia faba equina) are best; they grow vigorously, are little liable to mould, and do not elongate the hypocotyl and raise the cotyledons in germination, a point of much importance in most experiments on geotropism. Windsor or broad beans (Vicia faba) are also good, but germinate more slowly.

Tradescantia for protoplasm in stamen-hairs. Tradescantia virginica is best, but blossoms in spring and summer. It may be kept in blossom and in condition for use in September, October, and November by cutting the plants back to near the ground before they blossom in June, when they will come up again and blossom in the fall. If surrounded by a frame and covered by a sash on cool nights, they may be kept in good condition up to the end of November. Tradescantia zebrina, the Wandering Jew, grown in nearly all greenhouses, and blossoming most of the time, is nearly as good. But the hairs of gourds may be used instead (see page 51).

Nitella, for protoplasmic movement, etc. May be kept in good condition in large dishes in the greenhouse all winter; must not be given too much light. Chara is nearly as good.

Ricinus, for absorption, root-pressure, etc., should be planted and raised into 4-inch pots four weeks before needed.

Tropæolum majus, the Garden Nasturtium, should be propagated from cuttings six weeks before needed. These plants are easily grown and most useful for many purposes. They should be always in stock.

Sensitive plants should be grown, at a temperature of about 70°, in a sandy soil from seed planted five months before they are needed.

Coleus is best propagated from cuttings in a warm house five weeks before needed.

Impatiens Sultani, a useful plant, grown from seed needs two to three months to bring it into 4-inch pots.

Melothria punctata, used for its tendrils, is kept propagated by cuttings and can be rooted and brought into 3-inch pots in five to six weeks. Echinocystis is also excellent, for outdoor work.

E. PERSONAL EFFECTS OF EACH STUDENT.

Note-book of 200 pages, as described on page 14.
Colored pencils.
Liquid India ink and fine mapping-pen.
Towel, sponge.

5. MANIPULATION.

Much of the manipulation required is simply that learned in every elementary course in Chemistry, but for completeness the most important processes will here be described. Special methods used only once or twice in the course will be described under the particular experiments.

To Cut Glass Tubing. If under about 1 cm. diameter, file a shallow nick at spot to be broken ; turn this from you, and, grasping the tube firmly with thumbs and forefingers each side of the nick, pull apart the two ends and bend the nick sharply away from you, when the tube will break across. If slightly over 1 cm. diameter, a deeper nick will allow it to be broken in this way ; but if much over, or if a vial or a bottle is to be broken, the following method is efficient. File a nick across where the desired line of separation is, tapering the nick each way. Wrap around the tube two long strips of wet filter-paper parallel with the desired line of separation, each a mm. from it in thin tubing and farther in thick. Hold the nick in the hottest part of a Bunsen flame until it cracks cleanly across. Or, file a groove all around the place to be broken, making it deeper in one part. Hold this spot in the hottest part of a fine blowpipe flame, when the glass will crack along the groove. A very hot iron led along the path to be broken is said to be effective. For large glass tubing a very efficient special cutter may be purchased.

To Bend Glass Tubing. For smooth gradual bends, use the ordinary fish-tail luminous (or the Bunsen wing-top) flame; revolve the tube lengthwise in the flame for a very gradual bend, and obliquely for less gradual bends. For making special shapes, bend or mould the hot glass with a cold iron.

To Make Capillary Tubes and Rods. Hold the smallest available glass tubing in either flame, revolving it until soft, and then pull apart the two ends. The fineness of the tubing can be controlled by the degree of heating and rapidity of the drawing-out. The same method applies to solid capillary filaments, though for most purposes the lighter tubes are preferable. To keep the capillaries straight, pull up and down, not to right and left.

To Smooth Rough Glass Ends and Edges. Hold the rough parts in the Bunsen flame until they fuse smooth. Small tubing may be thrust at once into the flame, but large tubing and glass plates, etc., must be brought into it very cautiously. The rough ends of fine capillary tubes (which may be cut across with scissors) may be smoothed by careful rubbing on an oil-stone, or on a piece of ground glass, or by cautious fusing.

To Seal the End of a Glass Tube. If held in a hot Bunsen flame (the tip of the inner cone is the hottest part) and revolved, it will fuse itself together, closing perfectly. Or the tube may be drawn out to a capillary point, and after it is adjusted in some desired position, the capillary point may be sealed in a moment by a gas- or spirit-lamp flame.

To Prevent Evaporation from a Free Water or Other Surface. If a water-surface, place on it a film of oil, such as paraffine oil (or some other which will not become gummy or rancid on exposure to air), which is a very perfect preventive. If a flower-pot or similar object, cover it with dentist's rubber sheeting, which is a very good though not perfect preventive of evaporation. It is said also that cleaning, drying, and painting the surface of the pot with melted paraffine (which may easily be peeled off after the experiment) efficiently prevents evaporation.

To Graduate a Tube Temporarily. If only a relative graduation is needed, proceed thus. Stretch a fine thread by a wire spring, much as a bent bow is strung, and on it place a drop of liquid India ink, which will spread by capillarity; place the tube beside a mm. measure, and, touching the thread to the glass, even marks of any desired fineness may be made at any desired intervals, or a pasteboard mm. scale, or a paper mm. scale on a wooden strip, may be wired to the tube. If a tube graduated in definite units is needed, it is better to use a ready-graduated burette or pipette, in the absence of which a measured quantity of water is to be drawn into the tube by sucking on a rubber tube forced over its upper end; the top and bottom positions of the liquid are to be marked, and intermediate capacities obtained and marked by the mm. scale and thread as above. Tubes of disabled thermometers (or, better, the scales of milkglass-scale thermometers) make good graduates if wired to the liquid-tube, or a wooden mm. measure may be wired firmly to the tube.

To Graduate a Tube Permanently. Make the needed marks with a fine file, and rub white lead into them; or plaster of Paris containing some insoluble color (as carmine) may be thus used.

To Start a Siphon. Use a rubber tube, and immerse it in the liquid, allowing it to fill; pinch together one end so the air cannot enter, and lift this end out over the edge of the vessel and let it fall below the inner level; let the pinched end open, when the flow will start. Or fill a glass siphon, and hold the fingers over the two ends until it is in position.

To Make a Water-tight Joint between a Glass Tube and a Cut Plant. If the outer diameter of tube and plant is the same, slip a piece an inch long of soft rubber tubing of somewhat smaller diameter over both; it may be well (but not necessary) to tie the tube to the plant with a stretched rubber band, and to tie it to the glass tube in the same way, or with wire. If tube and plant are of different diameters, place upon the smaller a half-inch of soft rubber tubing of a size proper to make it nearly the diameter of the other; then slip a one-inch piece over them both as above described. When relative size of plant and tube permit it, it is well to fit a thin rubber tube over the plant, letting it project about 3 mm., and then push the tube over it so the plant and rubber fit like a cork. Sometimes it is convenient to use a wax or cement instead of the above joint. For this Detmer recommends a compound of 2 parts olive oil, 1 part mutton suet and 1 part wax, the proportions being varied somewhat according to weather, more oil being used at lower temperatures. Tire-tape alone often makes a good joint.

To Make a Water- and Pressure-tight Joint between a Cut Stem and a Glass Tube. Prepare a water-tight joint precisely as described in the preceding; then wind it tightly and carefully with several (4 or 5) turns of electrician's tape or tire-tape. A stout rubber stopper makes a good joint tight to moderate pressures.

To Tie Joints, etc. In all cases where pressure does not matter, as in fastening rubber to glass tubes, copper wire twisted tight by tweezers is best. A single turn of the wire, with the ends pulled out by pliers until the wire sinks all around into the rubber and then twisted twice, makes the best joint. Where pressure must not be too great the copper wire is unsafe, and thread must be used, or, better, stretched rubber bands which may be tied like string.

To Make Small Joints, Stop-cocks, etc., Air-tight. Where rubber tubing wired on is impracticable, a cement may be used composed of a mixture of vaseline and beeswax, a larger proportion of the latter being used for higher temperatures.

To Make a Tight Joint between Glass Tubing of Different Sizes. Draw out the end of the smaller piece so that it tapers somewhat; on this part place a piece of rubber tubing, and force this rubber-covered part in the manner of a rubber stopper into the larger tube.

To Cut or Bore Rubber. This is much easier if instrument and rubber are kept wet, either with water or, much better, with solution of potash.

To Bore Corks. This is done by use of the usual brass cork-borers (a sharpener for these should often be used); round files may be used for some odd forms or sizes.

To Improve Corks. Corks should always be softened with the cork-presser (for which the rotary forms are particularly good) before using, for both their power of holding in the bottle and of stopping passage of liquids is thereby increased.

To Make Ordinary Corks Air-tight. To prevent air-passage it is better to use rubber corks, but ordinary corks may be made fairly impervious to air by boiling them with paraffine or smearing them with vaseline.

To Test the Tightness of Joints to Gas-passage, etc. (see Fig. 7). Prepare a glass tube 1 cm. or less in outside diameter, and about 110 cm. in length. Bend 6 or 8 cm. at one end around in a U curve until it is parallel with the remainder, and to this attach by a stout rubber tube 3 cm. long, tightly wired on, a glass stop-cock; to the other end fix similarly a glass funnel. Set the whole firmly in a fixed vertical position, funnel upwards. The joint, tube, or other part to be tested is then attached, by a stout rubber tube (wired at both ends), to the stop-cock tube, and mercury is poured through the funnel until the long tube is filled; the mercury will then exert about an atmosphere of pressure upon the air in the part being tested, and this pressure may be left on for any desired time. To remove the part, close the stop-cock and place a dish beneath the tube to catch escaping mercury; the joint may now be loosened and the part removed above the stop-cock. Joints which are often tight to such pressure for a few hours or days, often yield to it afterwards, particularly in the case of waxed stop-cocks. In comparing readings of the compression at different times, allowance must be made for expansion of the compressed air under changes of temperature.

To Cleanse and Sterilize Germinators, Flower-pots, etc. They may be cleansed by rubbing in water with a stiff brush inside and out. For very delicate work they may be boiled first in water containing a weak solution of potash, and next in water containing a little hydrochloric acid, and next in pure water. They may be sterilized by dry steam in a sterilizer of the Arnold type, or, of course, by boiling in water.

To Clean Mercury. An excellent method * (Fig. 7) is to keep it in a vessel which has a glass stop-cock below from which it may be drawn off

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* Given by Arthur in the Botanical Gazette, **22**, 471. He also figures an excellent receptacle for holding the mercury. See also Detmer, 42, 43.

and to place on it a cm. of sulphuric acid and mercurous sulphate. After use the mercury may be poured in at the top, and may be drawn off dry and pure below.

To Remove Carbon Dioxide from a Closed Space. Hold the receptacle containing the gas over a small dish of water, and add to the latter some caustic potash, when the liquid will rise, absorbing the gas. This method also affords a fair test of the quantity of the gas contained in a given space.

To Test for the Presence of Carbon Dioxide. If in considerable quantity, the method described for its absorption may be used. If in smaller quantity, some filtered lime, or baryta, water brought into contact with it will turn milky if the gas is present. In air containing about three per cent or more of the gas a candle will not burn.

To Generate Carbon Dioxide. Prepare a large wide-mouthed bottle as shown in Fig. 7, with a tight cork through which passes a thistle-tube and a plain tube. Place pieces of marble, limestone, or chalk in it and cover them with water, below the surface of which the thistle-tube is to dip. From the plain tube, which ends just below the cork, lead a rubber tube to the place where the gas is needed, and pour a little hydrochloric acid through the thistle-tube, when the carbon dioxide will come off copiously, though at first much mixed with air. For most purposes it is well to add a wash-bottle to prevent acid going over with the gas. For this use a small bottle with a tight cork through which pass two glass tubes, one dipping below the surface of an inch of water. Connect the plain tube of generator and the long tube of the bottle by a rubber tube, and lead a rubber tube from the short tube of the bottle to the place of use of the gas. In using the apparatus, time must be allowed for the gas to drive out the air. For some investigation purposes this arrangement does not give a sufficiently pure gas, which may be obtained as described in all works on chemistry.

To Test for the Presence of Oxygen. If the quantity is proportionally much larger than occurs in the atmosphere, a glowing splinter thrust into it will burst into flame. Or phosphorus (thin sticks especially useful for the purpose are obtainable) will give off white fumes in a space containing oxygen; if over water, these fumes are absorbed by it, allowing it to rise into the vessel. Or, the pyrogallic method described in the next section may be used. The phosphorus must be kept always under water when not in use, and must not be touched by the hands. In testing it may best be used in a cage of wire netting on the end of a copper wire (Fig. 7). The use of several thin sticks at once will give the oxygen test very quickly.

To Remove Oxygen from a Closed Space. Plunge the open mouth of the vessel containing it just under the surface of water in a small dish, and add to the water enough well-mixed caustic potash and pyrogallic acid to make a concentrated solution. The mixture will absorb the oxygen and rise in the vessel. Where practicable it is a good plan to place the pyrogallic acid in the closed space (see page 97). The method, of course, also gives the amount of oxygen contained in a given vessel. Or, the use of phosphorus, as described in the preceding paragraph, also accomplishes this end, but it cannot be used with moist seeds, for it injures these.

To Render Thread Non-hygroscopic. Use a silk thread and thoroughly wax it by drawing it several times over a lump of beeswax; this will render it nearly but not entirely non-hygroscopic.*

To Transfer Tubes, etc., with Open Ends in Liquids to Other Liquids. Prepare a small dish (as the lower end of a vial, removed by the method earlier described) large enough to fit easily over the lower end of the tube; fasten to it a wire handle; slip the dish beneath the end of the tube, and lift it from the liquid; it will be sealed from air by the liquid of the dish, and may thus be lowered into the new liquid, and the dish removed.

To Keep Chemicals Pure. Make it a rule never to pour back chemicals into the bottle from which they were taken.

To Remove Stuck Glass Stoppers from Bottles. If the top of the stopper is flat, it may be placed in the hinge-crack of a door partly closed upon it, when a gentle turning of the bottle often loosens the stopper. Or, holding the bottle suspended from the stopper a half inch above the table, tap the stopper with a wooden hammer-handle or equivalent. Or, twist a cotton rag into a spiral roll several inches long, and dip it into boiling water excepting the ends, which are held; twist it quickly around the neck of the bottle, which swells the neck and usually loosens the stopper.

To Clean Sediment from a Bottle. Place shot or coarsely crushed glass (fragments of tubing) in the bottle and thoroughly shake and circulate it with water.

To Bore Holes Through Glass. Use the end of a round file; keep it wet with turpentine and camphor and rotate, using a carpenter's brace,

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* The hygroscopic variation in length of even an unwaxed thread is but slight. I have carefully experimented upon this point with silk sewing-threads a meter long hung in a tube into which moist and dry air could be drawn. The total range of variation in length in an unwaxed thread was 1.1 cm., that is 1.1%; in a thread waxed in the ordinary way it was .5 cm., i.e. one half of one per cent, while with a thread boiled in wax .85 cm., i.e. .85 of one per cent. Threads of similar length hung in an open room and read carefully during different kinds of weather gave much less variation. Hence in any apparatus in which threads must be used, waxed silk threads give so small an error due to hygroscopicity that it may be neglected, especially if as short threads as possible are always employed.

pressing lightly against the glass. Or, emery flour and camphor are said to be excellent. A drill may be used, or even, it is said, a brass cork-borer held in position in a hole bored in a wooden block cemented to the glass.

To Label a Glass-stoppered Bottle. Write with pencil upon the ground glass of the stopper and it will show through the neck. Also for temporarily labelling glassware generally a special colored pencil which marks on glass is obtainable.

To Prevent Decay in Liquids. A 2% solution of formaline will effect this. In osmotic experiments it must be used in equal strength inside and outside the membrane (see page 62).

To Cork a Full Bottle or Test-tube. Force in the cork with a fine wire or thread between it and the neck (which will allow the escape of air) and later withdraw this thread.

To Diminish Wilting in Cut Plants. If shoots are bent over and cut under water, they will as a rule keep from wilting much longer than if cut in air. This is because ordinarily in vigorous plants the air in their ducts is rarefied, and hence more air rushes in from without when the cut is made, thus partially blocking the ducts. When the part below the cut is to be used for root-pressure, etc., this precaution is needless, as the escaping sap will force out the air.

To Give a Constant Water-supply to Flower-pots, Germinators, etc. Place these in a flat-bottomed saucer (indurated-fiber saucers are particularly good *). Take a wide-mouthed bottle, and select for it a cork of such a size that, when forced into the bottle, it leaves projecting above the neck a length of cork rather greater than the depth of water needed in the saucer to keep the given objects properly wet. Along the sides of this cork deeply file four grooves; fill the bottle with water, force in the cork, invert the bottle and stand it on the cork in the middle of the saucer (see Fig. 26). The water will run out through the grooves in the cork until it fills the saucer to the desired depth, after which the water will be held at that level as evaporation proceeds. If it is not convenient to place the bottle in the saucer, it may be placed on a support at a distance and a vent-tube led into the saucer with its end at the proper height above the bottom (Fig. 27).

To Germinate Seeds. Where only the germination of seeds, and not growth of seedlings, is desired, the best germinators are porous earthenware flower-pot saucers, covered with others like them and allowed to soak up water from beneath. If thoroughly sterilized by boiling to start with, the saucer filters out the spores from the water entering it, and seeds germinate to great perfection. Very excellent in place of the

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* Obtainable at small cost from dealers in seeds and gardeners' supplies. They are very useful and much lighter than flower-pot saucers.

saucers are the covered Zurich germinators, made for the purpose.* These germinators, or the saucers, standing in a fiber saucer with the water-bottle described in the preceding section, give an ideal germinating apparatus.

If young seedlings are required, they grow excellently in chopped sphagnum moss, completely freed from all lumps, in ventilated wooden boxes, and these may well have a sloping glass side that they may be used also for experiments in growth and geotropism of roots. An excellent box (shown in Fig. 30) is of thin wood, 8 inches long, by 6 broad and 5 deep, with a glass front sliding into a groove, and sloping at an angle of about 20°. The bottom has open slits and the whole is heavily painted. They cost in quantity made at a box factory about 12 cents each. Seeds germinate well in sawdust also (particularly if pure pine sawdust be used), but I find the sphagnum much superior, particularly the dead sphagnum obtained below the surface of bogs. The sloping glass so essential in observation of some physiological phenomena may, however, be very simply obtained by use of a large glass funnel in which the seeds may be grown, or even with a tumbler having sloping sides. For most purposes the seeds are more convenient for experiments if they are planted with hypocotyl pointing directly downwards.

To Darken Bell-jars, etc. Hoods can be made from black paper pinned into shape; or, very satisfactory hoods can be made from the felt drying-paper used in herbaria, which is stiff enough to keep its shape. It is rolled into a cylinder, a top to which is made by cutting the upper end into four strips, folding them inward and fastening with a paper-fastener. This felt paper is a good non-conductor, and useful on that account.

To Prepare a Moist Dark-chamber for Germination, etc. See the plan described later (on page 118).

To Prepare a Self- or Autographic Recorder for Small Movements. Wherever the movement is a direct one, and a thread may be attached to the moving part, the auxanometer wheel and cylinder later described (on page 103) may be used.

To Measure Small Downward Pressures. For this the mercury flotation apparatus later described (page 124) may be used.

To Expose Plant Parts to the Influence of Pure Colors. For this the compound color-chambers later described (page 108) may be used.

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* They may be bought in this country from A. H. Hewes & Co. of North Cambridge, Mass., at a cost of 3 cents each.

TO CONVERT METRIC INTO ORDINARY ENGLISH MEASURES, AND VICE VERSA.

A. METRIC TO ENGLISH.

The standard of the metric system is the meter, which is the one ten-millionth part of a meridian quadrant of the earth; its one-tenth part is the decimeter, the cube of which is the standard of capacity, the liter. The one one-hundredth part of the meter is the centimeter, and a cubic centimeter of pure water at its maximum density (4° C.) gives the gram, the standard of weight. Divisions of the standards are expressed by Latin prefixes, *deci, centi, milli,* while multiples are expressed by Greek prefixes, *deka, hecto, kilo, myria.*

LENGTH.

Metric Name.	Relation to Standard.	Abbreviation.	English Equivalent.	Approximately in English.
Kilometer	1000 meters	km.	1093.61 yards .62138 miles	$\frac{2}{3}$ of a mile
Meter	Standard	m.	39.37 inches 3.28 feet 1.094 yards	$39\frac{1}{3}$ inches
Decimeter	$\frac{1}{10}$ of a meter	dm.	3.937 inches	4 inches
Centimeter	$\frac{1}{100}$ of a meter	cm.	.3937 inches	$\frac{2}{5}$ of an inch
Millimeter	$\frac{1}{1000}$ of a meter	mm.	.03937 inches	$\frac{1}{25}$ of an inch
Micron or micro-millimeter	$\frac{1}{1000}$ of a millimeter	μ	.00003937 inches	$\frac{1}{25000}$ of an inch

CAPACITY.

Liter	Standard	l.	1.057 U. S. quarts 61.03 cubic inches	1 quart U. S. meas.
Cubic centimeter (milliliter)	$\frac{1}{1000}$ of a liter	cc.	.001057 U. S. quarts .06103 cubic inches .034 fluid ounces	$\frac{1}{1000}$ of a qt.

WEIGHT.

Kilogram	1000 grams	kg. or kilo.	2.205 lbs. 2 lbs. 3 oz. $4\frac{3}{8}$ dr. avoirdupois	$2\frac{1}{5}$ pounds
Gram	Standard	gm.	15.43 grains (avoir.) .0353 ounces (avoir.) .643 pennyweights Troy	$\frac{1}{28}$ of an oz.
Milligram	$\frac{1}{1000}$ of a gram	mg.	.01543 grains (avoird. or Troy)	

There are many other intermediate measures, and also square measures, but they are less used, and their capacities may readily be calculated from those given.

The assumed standard height of the mercury column at sea-level is 30 inches in English or 760 mm. in metric. The pressure of the mercury column (1 atmosphere) is approximately 15 lbs. (really $14\frac{7}{10}$ lbs.) to the square inch in English, and 1 kilogram (somewhat more) to the square centimeter in metric.

B. ENGLISH TO METRIC.

LENGTH.

English Name.	Metric Equivalent.
Mile	1.609 kilometers
Yard	.914 meters 91.44 centimeters
Foot	30.48 centimeters
Inch	2.54 centimeters 25.40 millimeters

CAPACITY.

English Name.	Metric Equivalent.
Quart (U. S.)	.946 liters 946.36 cu. centimeters
Pint (U. S.)	473.18 cubic centimeters
Gill (U. S.)	118.29 cubic centimeters
Fluid ounce	29.57 cubic centimeters
Cubic inch	16.39 cubic centimeters

WEIGHT.

English Name.	Metric Equivalent.
Pound (avoirdupois)	.4536 kilograms 453.59 grams
Ounce ··	28.35 grams
Grain ··	.0648 grams 64. 79 milligrams
Ounce (Troy)	31.103 grams
Pennyweight (Troy)	1.555 grams
Grain ·· (= avoird. grain)	.0648 grams 64.80 milligrams

TO CONVERT CENTIGRADE INTO FAHRENHEIT DEGREES, OR VICE VERSA.

Rule : To change Centigrade to Fahrenheit, multiply by 9/5 and add 32. To change Fahrenheit to Centigrade, subtract 32 and multiply by 5/9. Or, use the following table :

Centigrade.	Fahrenheit.	Centigrade.	Fahrenheit.	Centigrade.	Fahrenheit.	Centigrade.	Fahrenheit.	Centigrade.	Fahrenheit.
+100°	+212°	+46°	+114.8°	+28°	+82.4°	+10°	+50°	− 8°	+17.6°
90	194	45	113	27	80.6	9	48.2	− 9	15.8
80	176	44	111.2	26	78.8	8	46.4	−10	14
70	158	43	109.4	25	77	7	44.6	−11	12.2
60	140	42	107.6	24	75.2	6	42.8	−12	10.4
59	138.2	41	105.8	23	73.4	5	41	−13	8.6
58	136.4	40	104	22	71.6	4	39.2	−14	6.8
57	134.6	39	102.2	21	69.8	3	37.4	−15	5
56	132.8	38	100.4	20	68	2	35.6	−16	3.2
55	131.0	37	98.6	19	66.2	1	33.8	−17	1.4
54	129.2	36	96.8	18	64.4	0	32	−18	− 0.4
53	127.4	35	95	17	62.6	−1	30.2	−19	− 2.2
52	125.6	34	93.2	16	60.8	−2	28.4	−20	− 4
51	123.8	33	91.4	15	59	−3	26.6	−21	− 5.8
50	122	32	89.6	14	57.2	−4	24.8	−25	−13
49	120.2	31	87.8	13	55.4	−5	23	−30	−22
48	118.4	30	86	12	53.6	−6	21.2	−40	−40
47	116.6	29	84.2	11	51.8	−7	19.4	−50	−58

PART II.

OUTLINE OF A COURSE IN EXPERIMENTAL PLANT PHYSIOLOGY.

THE PHYSIOLOGY OF PLANTS.

OUTLINE OF THE COURSE.

PHYSIOLOGY, in its broad sense, includes all operations carried on by living matter. There is but one living substance known,—PROTOPLASM. Obviously the study of the physiological operations and processes of plants should be preceded by a study of the structure and properties of the substance which is the sole physical basis of life and its phenomena. Hence a complete treatment of our present subject involves two divisions, which, with their leading subdivisions, are as follows:

Division I. THE STRUCTURE AND PROPERTIES OF PROTOPLASM.

1. Its composition,—molar, mechanical, physical, and chemical.
2. Its relations to external conditions.
3. Its power of organism-building.

Division II. THE PHYSIOLOGICAL OPERATIONS OF PLANTS.

1. Nutrition.
2. Growth.
3. Reproduction.
4. Irritability.
5. Locomotion.
6. Protection.

DIVISION I.

THE STRUCTURE AND PROPERTIES OF PROTOPLASM.

Section 1. The Composition of Protoplasm,—molar, mechanical, physical, and chemical.

1. What is the molar composition (i.e., the form and size of the masses) of the living Protoplasm of Plants?

Your studies in previous courses have given you ample data for answering this question. Take time to think it over, recall and review your present knowledge, supplement it from books, lectures, or other available sources, and answer at your leisure.

Leave page 1 in your laboratory book for this.

2. What is the mechanical composition (i.e., what structures and differentiations has a single mass) of the living Protoplasm of Plants?

Answer by concise descriptions, illustrated by logically complete drawings, based upon a minute study of single protoplasts of (*a*) the stamen-hairs of *Tradescantia virginica*, and (*b*) the tip-cells of *Nitella*. Supplement by additional data from any available sources.

> *Select young fresh-looking hairs or tips, and mount in tap-water on a slide under a cover-glass. If tips are dirty, clean with camel's-hair brush. At present it is not necessary to pay any attention to details of the movement.*

Living Protoplasm, excellent for observation, may be found also in the leaf-cells of Elodea canadensis, in the hairs on the stems of tomato, squash, and some other gourds, Cypripedium spectabile, and doubtless of many other plants. It may be found also in some root-hairs, and in the hyphæ of some fungi. Practical directions for preparing these objects for examination are given by Strasburger in Chapter 3 of his "Kleines

Practicum" (English translation by Hillhouse, 5th ed. 1900, New York, Macmillan). On obtaining Tradescantia in autumn and winter see page 37.

Owing to the nearly uniform transparency of most of its essential parts, Protoplasm in a living state shows only partially its internal structure. To render this more fully visible, a protoplast must be carefully killed and differentially stained; this has been done in the mounted specimens supplied.

3. How much of the fundamental structure of Protoplasm can be shown by the best-prepared dead protoplasts?

Answer from a study of the preparations supplied, supplemented by other available data.

If students have already studied cytology, this need be but review otherwise cytological preparations should be used.

4. What is the physical composition (i.e., the texture, density, motility or rigidity, color and other optical properties) of the living Protoplasm of Plants?

Answer by concise descriptions made during your observations for Exercise 2 above.

5. What is the chemical composition of living Protoplasm?

Direct observation and experiment upon this most important subject is here impossible because of practical difficulties; answer from data gathered from lecture-notes and accessible books; express in a concise paragraph.

CORRELATED TOPICS.

(Important to completeness of the subject.)

History of our knowledge of Protoplasm.

Present distribution of Protoplasm in space and temperature.

Ontogeny of Protoplasm, and the origin and meaning of death.

Phylogeny of Protoplasm; theories as to its origin and its relations with non-living matter.

Interpretation of the visible structure of Protoplasm, and theories as to its ultra-visible and ultimate structure.

The protoplast and its "contents."

IMPORTANT LITERATURE.

The standard general works (especially Pfeffer's Physiology).

Wilson, E. B. Structure of Protoplasm. Science, **10,** 33, 1899.

Verworn, M. (translated by Lee). General Physiology, 55–136.

Goodale, G. L. Protoplasm and its History. Botanical Gazette, **14,** 235, 1889.

Bütschli, O. Protoplasm and Microscopic Foams. (Reviews in Nature, **48,** 595, 1893, and in Science, **2,** 893, 1895.)

Section 2. The Relations of Protoplasm to External Conditions.

In order to test the effects of external conditions and influences upon living Protoplasm, it is needful to use some method by which the responses to those conditions and influences may be made visible. The movement of circulation or rotation of the cytoplasm in the cells of certain plants is extremely sensitive to some external influences, and hence offers a convenient test. It is of course necessary first to become familiar with this movement as it occurs under normal conditions.

6. What is the general character, the extent, the constancy, the approximate rate, the places of greatest activity of the movement in the protoplasm of (a) the stamen-hair of Tradescantia virginica, and (b) the tip-cells of Nitella?

Answer by annotated diagrams and concise descriptions.

Mount on a slide in which a slight hollow is ground, or support the cover on tiny legs of wax, so that it may not press upon the object.

In what are the two alike as to the movement, and in what different?

What is your opinion as to the use or meaning of the movement?

Is this rapid movement of wide occurrence?

7. What is the exact rate of movement in these two plants under ordinary conditions?

Answer by precise quantitative measurements.

To make these it is necessary to observe how long it takes for some of the swiftest-moving granules in the Protoplasm to pass over a given space. Time may be measured by a metronome (see Fig. 8) ticking seconds, and space by an ocular micrometer, the relative value of the scale of the latter being first determined by use of a stage micrometer. The temperature of the room should be noted.

(*Cells showing the most active movement should be selected. To allow of comparison of results between students, measurements should all be expressed in the same units, preferably mm., per minute taken for the even degrees. Use flat mirror to avoid focusing of heat; do not pinch the cell with forceps; do not use material recently chilled by cold air.*)

8. What are the various classes of external influences which can be brought to bear upon living Protoplasm?

Answer by a classified table worked out by yourselves.

Of the various external influences which can readily be brought to bear upon Protoplasm, the most important are:

(1) Temperature changes.
(2) Light.
(3) Electricity.
(4) Mechanical Shock.
(5) Chemical Substances.*

9. What effect is produced upon Protoplasm, as manifested in its rate of movement, by changes of temperature?

Answer by Experiment 1.

EXPERIMENT 1. To determine this, some method is needful by which the temperature of a given protoplast may be raised and lowered at will, while the rate of cytoplasmic movement is determined at each degree. Measurements may be made as already done under Exercise 7; temperature may be controlled by the temperature-stage

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*Gravitation and some others are here omitted, because this section is not concerned with active responses to external influences acting as stimuli (which are later to be studied under Irritability), but with the direct (mechanical, physical, and chemical) effects produced by outside influences upon protoplasm. The distinction is important, though of course the two merge into one another, and are often indistinguishable.

Carefully select an active, clean, tip-cell of Nitella, and mount in water so that the cover-glass will not press upon it. Place on the temperature-stage and warm the latter slowly and evenly by use of a spirit-lamp. Determine the rate of movement at regular and frequent intervals and note with care the exact point at which the movement is most active (the optimum) and that at which it ceases (the maximum). Later, with the same cell or one as nearly like it as possible, reduce the temperature by filling the water-box gradually with a mixture of ice and salt, observing and recording as before; note the point at which movement ceases (the minimum). Do not be content with a single observation, but try again and average.

(*Express results in mm. per minute for each two degrees, even numbers, of temperature, to allow comparison of results. The heating and cooling must be done slowly, else the sudden change acts as a shock, affecting the result. Use only the plane mirror, as the concave focuses some heat.*)

The results of these observations are to be expressed not only in tables of figures, but also in curves (polygons) the points of which are established by the intersection of ordinates expressing rate of movement with abscissæ expressing degrees of temperature. (On this, see page 18.) On the same sheet with your individual record should be plotted the class average polygon. Comparison of this with your own will give some idea of the probable error in your results.

An efficient stage, represented in Fig. 8, may be made at small cost (about $1.50) by any tinsmith. It is of sheet copper, one-sixteenth of an inch thick, of the breadth of a microscope-stage, rolled over as shown by the figure to make a chamber for a thermometer and another for a three-inch slide, with holes between objective and mirror. Forward it dips down to enter a shallow (one inch deep) tin box which hangs from it by one cross-wire and two stubs, as shown in the figure, this arrangement allowing its removal. Both stage and box taper forward to one inch and one and one-half inches respectively in breadth, a feature not necessary but useful as diminishing the leverage of the box when full. A battery-clamp, properly filed, holds the apparatus to the stage of the microscope, while a mat of felt between prevents conduction of heat to or from the instrument. A bent thermometer, as shown in the figure, may be used, but the straight ones needed in the laboratory for other purposes are about as good. To raise the temperature, the box is nearly filled with water and heated by a spirit-lamp; to lower it, the box is gradually filled with ice and salt.*

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* Very careful tests of the accuracy of this stage have been made in comparison

Temperature-stages may be purchased at prices from $7.00 upwards. Various forms are figured in Goodale's Physiology, page 202, in Detmer. 421, in Verworn, 392. Very elaborate forms have been devised for special researches.

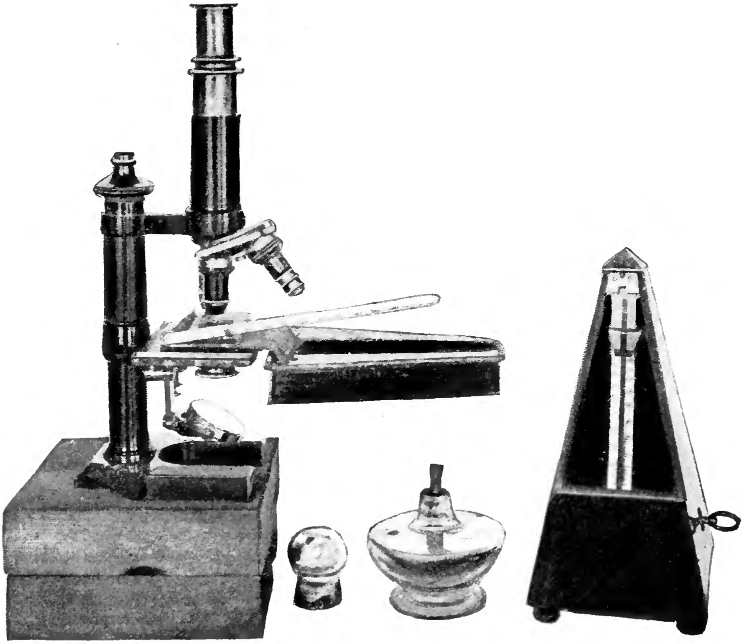

FIG. 8.—TEMPERATURE-STAGE AND METRONOME.
One-fourth the true size.

10. What effect is produced upon Protoplasm, as manifested in its rate of movement, by presence or absence of light, and by variations in its intensity?

Answer from the results of simple experiments invented by yourselves (call them Experiment 2).

Be careful to avoid the influence of variations in heat.

with other forms, with the result that it is found to be at least as accurate as any of those made on the principle of warming a metal plate (while superior to most of them is the ease and convenience with which temperatures may be reduced to zero), and nearly, if not quite, as accurate as those using a circulation of hot water.

11. What effect is produced upon Protoplasm, as manifested in its rate of movement, by electrical currents?

Answer by Experiment 3.

EXPERIMENT 3. To determine this, it is necessary to apply to the living protoplast electrical currents which may be controlled in strength and duration; this can be done by use of a simple electrical slide in circuit with one or more battery-cells and a circuit-closer, shown in Fig. 9. For induction-currents a small coil is to be introduced.

Place a tip-cell of Nitella upon the electrical slide, and test the effect upon the movement in its cytoplasm of (*a*) a single cell, (*b*) two and (*c*) three cells, each applied (1) instantaneously, (2) for a second, (3) two seconds, (4) three, (5) five, (6) ten seconds, and if necessary longer.

(*The ends of the piece of Nitella must be in contact with bits of tin foil which are in contact with the clips and wires.*)

Try also the effect of an induced current from an induction-coil.

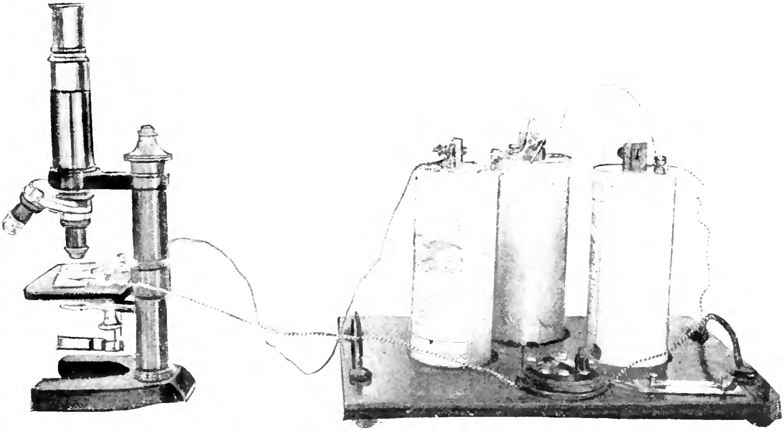

FIG. 9.—ELECTRICAL STAGE AND BATTERY.
One-fourth the true size.

Is it the continued action of the current which produces the effect, or the making and breaking of the circuit?

Since the plant is never exposed to this influence in nature, the response cannot be adaptive.

What then is the probable explanation of its behavior?

Ordinary dry battery-cells, with any quick-acting circuit-closer, are ample, and for convenience may be arranged as shown in Fig. 9. For an induction-coil, any small form (borrowable usually from the Department of Physics) will do.

An inexpensive electrical slide may be purchased in the Arthur Apparatus (see page 32), or may be made by cementing small binding-screws and strips of brass to an ordinary slide by sealing-wax, or yet more simply as follows (see Fig. 9): The clips belonging to the microscope-stage are insulated by pushing them into place surrounded by thin silk or rubber; their free ends are then brought within half of an inch of one another over an ordinary slide; thin strips of tin-foil are placed beneath them and the ends brought but a sixteenth of an inch apart; the Nitella cell is then placed with its ends on the tin-foil poles, is covered with water and a cover-glass, and the whole is ready for use. After the exact temperature-measurements of Exercise 9 it does not seem profitable to take time for very detailed quantitative measurements of effects of electricity, though this may readily be done by aid of electrometers.

12. What effect is produced upon Protoplasm, as manifested in its rate of movement, by mechanical shock?

Answer from the results of simple experiments invented by yourselves (call them Experiment 4).

Remember the golden rule of experiment,—to alter but a single condition.

13. What effects are produced upon living Protoplasm by chemical substances?

This subject, though of much importance, cannot here be taken up practically, and it is to be worked out from your various sources of information and expressed concisely in a paragraph. It is necessary to distinguish very clearly between the direct chemical effects produced by the chemicals upon the Protoplasm, and the irritable responses produced by chemicals acting as stimuli. The former alone are to be discussed here, while the latter will be studied later under Irritability.

There is one phase of the subject not difficult of experiment, i.e., the effects of anæsthetics, and of other gases, upon protoplasm as shown by the cytoplasmic movement. Full directions for this are given by Detmer, 422, and by Darwin and Acton, 18. An excellent gas-slide for the purpose is supplied in the Arthur apparatus.

Experiments on chemotropism will be referred to under Irritability.

CORRELATED TOPICS.

Summary of the distinctive properties of Protoplasm.

Distinction between the physical and the "vital" properties of Protoplasm.

Difference between the external influences acting (*a*) mechanically, (*b*) tonically, and (*c*) as stimuli.

Nature of the release of energy causing movement in the organism.

Ecological aspects of direct effects of light and heat upon Protoplasm.

Effect of varying quantities of water upon the resistance of Protoplasm to heat and cold.

Desiccation of Protoplasm, and its ecological value.

IMPORTANT LITERATURE.

The standard general works.

Davenport, C. B. Comparative Morphology, Parts I and II. (New York, Macmillan.)

Thurston, R. H. The Animal as a Machine and Prime Mover. Science, **1**, 365, 1895.

Hörmann, G. Studien über die Protoplasmaströmung bei den Characeen. Jena, 1898.

Loew, O. The Energy of Living Protoplasm. London, 1893.

—— The Proteids of Living Matter. Science, **11**, 930.

Section 3. The Power of Organism-building by Protoplasm.

Up to this point you have studied Protoplasm as it occurs and works in single protoplasts. But, of course, Protoplasm is not confined to these, but builds itself into great many-celled organisms. Since, however, Protoplasm is a soft substance possessing no strength for resisting the great mechanical strains to which large organisms are necessarily subject, it must be supported by a firm skeleton built by itself. This skeleton must permit the primary functions of the Protoplasm to go on, and allow for the several secondary needs of the organism. How is such a skeleton built?

14. What primary principle determines in multicellular plants the general size of the Cell?

How do protoplasmic masses act when they grow to the limit of the normal cell-size?

Answer from earlier studies or other sources of information.

15. How are the different Protoplasts of one organism kept in physiological continuity?

Answer from a review of your knowledge of continuity of Protoplasm, and from your other sources of information.

16. What are the main principles upon which a multicellular mass of Protoplasm builds its skeleton (in Plants)?

This subject is supposed to have been covered by your earlier studies in anatomy, and should need simply review from the physiological point of view. It should be worked out here in synoptical paragraphs, illustrated by diagrammatic drawings. Following are the most important topics:

a. What is the difference in the principle of skeleton-building in the higher plants as compared with the higher animals?
b. How is the cell-wall substance built by the Protoplasm?
c. What is the physical and chemical composition of cell-walls?
d. What shapes, thicknesses, and sizes may cell-walls assume?
e. How do tissues and organs arise?

17. Write, in your Laboratory books, a synoptical essay upon THE STRUCTURE AND PROPERTIES OF PROTOPLASM. It is to consist of a logically arranged and properly proportioned synopsis of the subject deduced from all of your sources of information. It should be prefaced by a tabular outline of contents and should not exceed six hundred words in length.

LITERATURE.

The standard work on this subject is Haberlandt's "Physiologische Pflanzenanatomie." But the general works, especially Strasburger's "Text-book," contain sections upon it.

DIVISION II.

THE PHYSIOLOGICAL OPERATIONS OF PLANTS.

Section 1. The Nutrition of Plants.

A. *Absorption (a) of Water and Dissolved Minerals.*

It is a familiar fact that water and minerals are absorbed by the higher plants through their roots. It is needful therefore to begin a study of absorption by an investigation into the structure of the absorbing parts of roots.

18. What is the structure of the absorbing part of the root of a typical land plant?

I.e., what is the structure of the tip, of the hairs, of the tracheæ? What is the distribution of the ducts, and the relation of the hairs with them? What is the relation of the root-hairs to the soil-particles?

Answer from a study of the roots of mustard and oats, grown both in germinators and in soil. Construct a diagram of the structure of the young root as an absorbing apparatus.

Root-tips and hairs in perfect condition may be obtained thus: in a small, very porous flower-pot saucer (or in a Zurich germinator) place seeds of mustard, soaked an hour or two, and of oats, soaked overnight; cover with a similar saucer, and set in a dish of water deep enough to keep the inside of the seed saucer always moist, though not wet. (See page 44.) Three days will bring them to perfection.*

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* An extremely easy and very effective method of obtaining perfect root-hairs for general student work is the following: Take a flower-pot, any size (say five-inch); cork the hole in the bottom; throw into it with some force a small handful of mustard-seeds soaked a few hours; the seeds with their mucilage will stick to the pot; invert the pot and set in a saucer of water for two or three days.

(*If the mustard roots are placed in strong solution of potash, they will show the ducts and growing point without sectioning; the protoplasm in the hairs may be made more plain by plasmolysis with a weak* (5%) *solution of salt; the important transverse distribution of ducts and sieve elements can be seen only in cross-sections.*)

Your studies of the structure of the roots (under 18) show that the special water-absorbing parts of plants, i.e., the hairs on the roots, are not open tubes, but closed sacs. Water must therefore enter through imperforate membranes, and hence it is necessary to consider first the physics of the transfer of liquids through membranes, i.e., the physical process of *Osmosis*, involving *Diffusion.*

19. What is the nature of Diffusion?

Answer by Experiment 5.

EXPERIMENT 5. Fill with water an erect test-tube, or equivalent, placed where it cannot be jarred, Drop quickly to the bottom a piece of solid fuchsin, and observe the diffusion of the color. Form a consistent mental picture of the direction of action and character of the energy involved, and indicate these in a diagram.*

(*Keep under as constant temperature as possible, so that the diffusion may not be too much influenced by convection currents.*)

20. What is the nature of diffusion through a membrane, i.e., of Osmosis?

Answer by aid of Experiment 6.

EXPERIMENT 6. For the study of this subject it is needful to use a membrane capable of being wetted by two liquids placed on opposite sides of it, and one at least of which must contain some crystallizable substance in solution; arrangements should be made, also, to measure any quantitative differences in the passage of the liquids through the membrane. Construct an osmometer as follows (see Fig. 10): Over the lower end of a burette, or other calibrated tube, 16 mm. in diameter, slip the end of a soaked diffusion-shell of 16 mm. diameter, and tie tightly with waxed thread. Fill cup and burette to the zero-mark with molasses (i.e., a colored solution of sugar) and immerse them to the zero-mark in a large dish of pure water. Cover both liquids with a film of oil, and add to both enough formaline to make a 2% solution. Observe and record frequently; calculate and express in a curve the quantitative changes

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* On this consult earlier, page 19.

in the liquid levels. Form a clear mental picture of the molecular processes involved and of the energy concerned, and express in a diagram.

(*Pour in the molasses through a small funnel not allowed to drip against the inside of the burette. If the liquid threatens to overflow, add other burettes or tubes with rubber connections.*)

For this experiment burettes specially made, 16 mm. external diameter, graduated to 50 cc., with 2 cm. of tube above and below the graduation, are best; they are not expensive, and they may be used for several other purposes. Or, the bottom may be removed from one of the ordinary kind. The diffusion-shell (made by Schleicher and Schuell, and supplied at low cost by Eimer & Amend of New York) is very efficient and convenient. But other membranes (such as pig's bladder, bookbinder's parchment, or even parchment paper, obtainable at all chemical

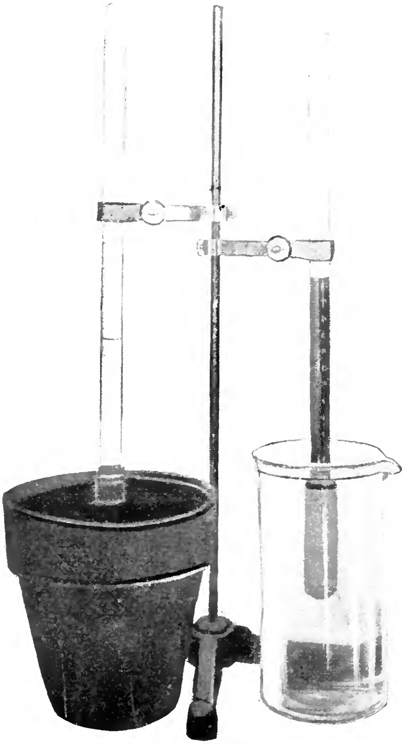

Fig. 10. — Osmometers.
One-fourth the true size.

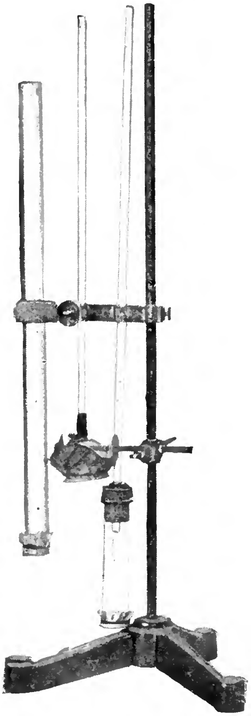

Fig. 11.—Simple Osmometers.
One-fourth the true size.

supply stores) work equally well. When thoroughly soaked they are to be tied over the lower end of the burette, but, having little surface, they work far more slowly than the shell. Various simple forms of osmometers are shown in Fig. 11. The advantage of the burette is that it allows exact measurements. A very simple and effective form using parchment-paper tubing is described by MacDougal in Journal of Applied Microscopy, 1, 56. It is especially valuable for class demonstration. Diffusion-shells of 40 mm. diameter are obtainable.

The osmometer shown in Fig. 10 can easily be made autographic by use of a frictionless float (see page 124) on the liquid, which is connected by a thread with the wheel and recording cylinder later described (page 103).

Properly, to give a true measure of the osmosis in the preceding experiment the two liquids should be kept at the same level by constantly sinking the burette or raising the level outside, but practically this is unnecessary, especially as the error is in the direction of a lesser and not a greater result. The size of the outer vessel makes some difference in the result, for if small the sugar passing exosmotically to it from the cup rapidly raises its concentration towards that of the liquid in the cup, hence diminishing both the rate and the amount of the rise in the latter. But, as my experiments show, above a certain size (about that shown in Fig. 10) the result is not appreciably affected by the size of the outer vessel.

Of course many crystallizable substances besides sugar may be used, such as potassium nitrate, etc. The advantage of the sugar is that it is probably the chief substance effective in the endosmotic absorption of water by the root-hairs, though it is possible that potassium nitrate plays also a part in this process. A great advantage of the molasses is its color.

The rate of diffusion through a membrane, even the possibility of its occurrence at all, varies with the membrane and with the substances in solution. The parchment cup used in Experiment 6 was readily permeable by the water, minerals, and sugar. There are other membranes called semi-permeable, which allow water and some dissolved substances to pass, but not others.

21. What effect has a semi-permeable membrane upon Osmosis? Answer by Experiment 7.

EXPERIMENT 7. An excellent semi-permeable membrane is formed by a precipitation-film of copper ferrocyanide, which may be made as follows: In a large upright test-tube, or its equivalent, place a

5% solution of potassium ferrocyanide (using care, for it is poisonous); drop quickly into it a compact small lump of copper chloride (or copper sulphate), which should sink to the bottom. Observe carefully the growth of the membrane, and form a clear and consistent mental picture of the molecular processes and the energy involved. Express these in a diagram. (This is worth observing also upon a slide under the microscope.)

22. What is the physical explanation of the results of the following simple experiments?

EXPERIMENT 8. (*a*) In five test-tubes place respectively water, a 25%, a 50%, a 75%, and a 100% solution of a saturated solution of sugar. Drop one or two raisins into each, and add formaline to make a 2% solution to prevent fermentation.

(*b*) Fill a hollow cut in a piece of beet (or carrot) with dry sugar.

(*c*) Boil a piece of red beet for a few minutes, and then place it in fresh water. In another dish of water place a similar but unboiled piece. Observe the effect, after a few days, upon the water.

What is the explanation of the bursting or collapsing of fruits in preserving? Of the crispness of celery when placed in water?

What other every-day osmotic phenomena can you think of?

It is said that the exact relationship of the killing of the protoplasm to the release of the color in the experiment 8 *c* may be determined beautifully by using thin slices of beet under the microscope, and applying the heat by the temperature-stage.

23. Can an apparatus be constructed to imitate the principle of the physical process of water and mineral absorption by roots?

Answer by observation of the construction and working of the Pfeffer artificial cell.

Though simple in principle, it is practically difficult to set up for action; so, instead, read the account of it in Goodale, Physiology, 226–230, in careful comparison with the specimen on the table. Place a synoptical account of it in the note-book.

Construct two diagrams showing comparative structure and differences in operation of this apparatus and of the absorbing system of the root; especially note the duration of absorption in the two cases, and its determinants.

It is well to set up one of these cells for observation by the entire class. Very full and clear directions are given in the pages of Goodale cited above. It is rather too difficult for each student to set up separately unless time is abundant. It is shown in Fig. 12. Compare also Copeland, "An Artificial Endodermis Cell," Botanical Gazette, **29**, 437. I have had fair success in simplifying this apparatus as follows: A small porous cup (obtainable at trifling cost), about 40 mm. diameter and 60 mm. long, is thoroughly washed. A bottle just sliding inside it has its bottom removed and is then firmly sealed into the top of the cup by sealing-wax. The cup is then soaked in boiled water overnight. Inside of it is then placed a 3% solution of potassium ferrocyanide, and it is set in a dish of 3% copper chloride solution, the two liquids being at the same level, and left overnight. The cup is then emptied and filled with molasses to which a few drops of potassium ferrocyanide are added; a rubber stopper containing a long glass tube is then inserted into the neck of the bottle and the cup is plunged into water containing a little copper chloride. An ascent of the inner liquid follows, and no trace of the color of the molasses comes out into the outer vessel. Instead of the long open tube, a short closed graduated tube may be used and the pressure determined by the compression of the air above it by Boyle's law (see page 68). In preparing this the tube should be drawn to an open capillary point, and sealed when the liquid has reached the zero mark of the scale. The bougies of Pasteur filters make excellent cups, but are more expensive. In using all such cups it is necessary that the rubber stopper should not come into contact with the cup itself, since its yielding under pressure would leave the membrane discontinuous. Where it must touch the cup, the latter should have a layer of sealing-wax extending below the stopper. It is not difficult to seal the tube into the bottle or bougie with sealing-wax. (See Note 1 of the Addenda).

24. Do roots actually absorb water in quantity and pass it up stems?

Answer by observation of Experiment 9.

EXPERIMENT 9. This may be tested by cutting off all but the root and lower part of the stem from a vigorous plant and noting whether water is forced out of the cut surface by the roots. Select a vigorous single-stemmed herbaceous plant, and cut it off an inch from the ground. To the stump attach a burette by a water-tight joint (see page 40); fill the burette to 2 cm. above the zero mark with water (to allow measurement in case it drops), add a film of oil to prevent loss by evaporation, and note the rise or fall of the liquid. (See Fig. 10.) A Ricinus is very good for this experiment.

Compare this arrangement with that of Experiment 6.

Note that in one case we have innumerable tiny root-hairs, and in the other, as it were, a single gigantic one.

But what other differences exist between the two?

This root-absorption (not, of course, root-pressure) apparatus can easily be made autographic by placing a frictionless float (see page 124) on the liquid and connecting it with a magnifying wheel carrying a pen against the recording cylinder (see page 103). It is more convenient for this purpose to use a burette only half the usual length, that the threads may not need to be too long. To determine whether the internal processes show any periodicity independently of outside influences, the experiment should be carried on in the dark-chamber with as constant temperature as possible. Since watering the plant affects the process very much, the plant should either be watered well at the beginning of the experiment, and not again (evaporation being hindered by a wrapping of rubber or equivalent method see page 78, or else a self-regulating apparatus should be used see page 109). An eight-day clock should be used to turn the cylinder, so the record need not be disturbed during the experiment.

25. Do living roots exert active pressure in water absorption as the artificial cells already studied do?

Answer by Experiment 10.

EXPERIMENT 10. This may be tested by leading the water given off by the roots (as shown in the preceding experiment) into a gauge where its pressure, if any, may be measured. Select a vigorously growing potted plant, such as a Ricinus or Sunflower. Take a piece of glass tubing whose inner bore is about the diameter of the plant; some 15 cm. from the end draw it to a capillary open point as abrupt as possible (by use of a very small flame) and bent over at right angles (effected by using little heat and bending with an iron instrument). Attach this gauge by a water- and pressure-tight joint (see page 40) to the stump of the plant cut off an inch from the ground (see Fig. 12). Attach by wire to the tube a mm. scale (unless it is already graduated). Wait until the liquid inside the tube reaches the zero mark, when the capillary point is to be quickly sealed in the flame of a spirit-lamp, care being taken (by interposing a piece of cardboard) not to heat and expand the air in the tube. The ascent of the water is to be observed, and when its extreme height is reached, the pressure exerted by it upon the closed air is to be calculated by Boyle's law. The final reading should be made as nearly as possible at the same temperature at which the tube was sealed.*

A source of error in this experiment is the vapor-tension in the tube,

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* With such gauges as these, my students have often obtained with young Ricinus plants, in November, a root-pressure equal to an atmosphere.

but this is so slight as to be negligible for general work. (See Note 2 of the Addenda.) Another would be the heating and expansion of the air in sealing the tube, but this is very slight and almost entirely avoidable.

A method in some ways better is to use instead of this plain tube a stop-cock tube (shown in Fig. 12); it is used precisely as is the plain tube, except that water may be added at once to bring the liquid to the zero mark of the scale, when the stop-cock is to be closed; (any drop through negative pressure will compensate itself.) The objection to the use of these tubes, aside from the expense, is the considerable difficulty of making them tight to pressures from within. For a method of testing their tightness see page 41. Some of them will remain tight to an atmosphere of pressure for several days, but then will leak. They are tightest when very little of the stop-cock wax is used, and when the cocks are thoroughly screwed into place.

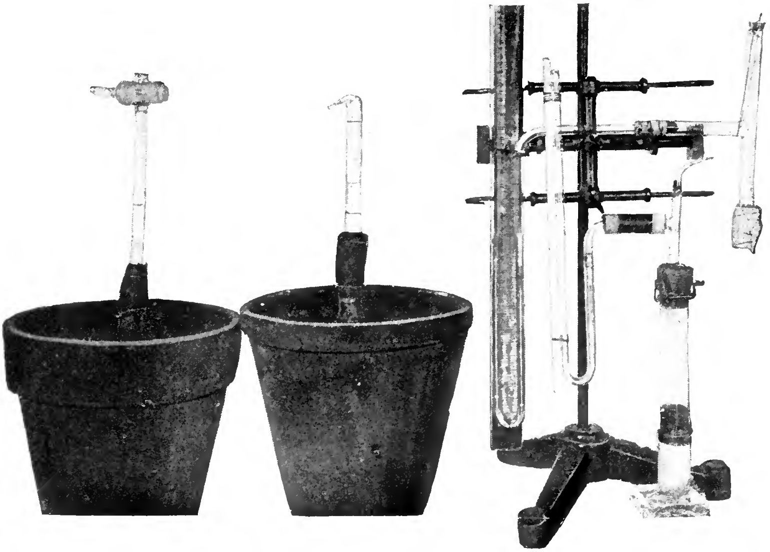

FIG. 12.—FORMS OF PRESSURE-GAUGES, FOR MEASURING ROOT AND OTHER OSMOTIC PRESSURES.
One-fourth the true size.

Boyle's (also called Mariotte's) law is this, that, the temperature being the same, the volume of a gas varies inversely as the pressure upon it. Thus if the volume of a gas becomes compressed to one-half its former volume, the pressure has been doubled; if compressed to $\frac{2}{3}$ its former volume, the pressure is $\frac{3}{2}$, i e., half as much again as at the start. If the liquid in the gauge above described rises so as to compress the volume of air to $\frac{3}{4}$ the volume it has at the start, the pressure must be $\frac{4}{3}$ of what it was at the start. But it was one atmosphere at the start, and hence the additional pressure exerted by the liquid is $\frac{4}{3}$ less $\frac{3}{3}$, that is $\frac{1}{3}$ of an atmosphere. Since an atmosphere of pressure is roughly 15 pounds to the square inch, the liquid is exerting a pressure of about 5 pounds to the square inch. The temperatures should be about the same when the readings are taken at the beginning and end of the experiment. Boyle's law is not strictly true for damp air, though nearly enough so for rough work. Greater exactness in the readings of the above gauges can be obtained by making them in two pieces, a lower short piece attached to the plant, which is then allowed to stand until this piece is partially filled with water and all air-bubbles have escaped; a film of oil is then placed on the water, the upper piece, thoroughly dried, is then attached by a pressure-tight joint and sealed. The oil keeps the air in the tube fairly dry.

Another method of measuring root-pressures is Pfeffer's (given in Detmer, 198). In using all such apparatus it is necessary to keep the tubes as small as conveniently possible, because the *volume* of water given off is often small even though its *pressure* may be high; if the gauge is large, the volume of water given off may not be sufficiently large to push the index surface high enough to register the true *pressure* under which it is being given off. The open S-shaped tube containing mercury, figured in many books, I find rather impracticable as usually recommended, partly because it is difficult to fill the space between plant and mercury with water without admitting air-bubbles and disturbing the pressure levels to start with, partly because the lower arm of the tube (that dipping below the top of the S) must be a very inconvenient length in order to register a high pressure (thus it must be over 38 cm. long to register one atmosphere). Moreover, as often figured, the tube is so large in diameter that the plant could not give off quantity enough to push the mercury high enough to register the actual pressure under which the liquid is being given off, but this of course could be overcome by using a tube of narrow bore. A modification of this arrangement, shown in Fig. 12 on the right, works fairly well. The manometer tube is sealed (and pressure calculated by Boyle's law), while the vertical corked tube allows all air to be removed between mercury and plant. (See Note 3 of the Addenda.)

A self-registering apparatus for measuring root-pressure is described by Thomas, in Botanical Gazette, **17**, 212.

The large pressures shown by the preceding experiments to result from osmotic absorption, suggest that elastic cells must be stretched by such pressure and hence acquire turgidity and therefore considerable mechanical rigidity; and also that the rigidity of delicate plant parts, lacking thick-walled elements, may be due to this cause.

26. Can the turgidity resulting from osmotic pressure afford mechanical rigidity?

Answer by Experiments 11 and 12.

EXPERIMENT 11. To test this an apparatus should be constructed which is flaccid to begin with, but capable of absorbing water osmotically. This can be done thus: Take a piece of parchment-paper tubing 40 mm. diameter and 15 cm. long; soak it well; gather one end into close folds and tie tightly with waxed thread; fill nearly full with molasses, gather the open end into folds, squeeze until the molasses begins to run out (to expel air) and tie tightly with thread. (Or, the ends may be tied tightly to large corks, preferably rubber, fitted into them.) The tube will now be so limp as to bend in the hand, lacking all rigidity. Place in a basin of water for a few hours and examine. Holding it in the air, prick the membrane with a pin. (Rubber bands joining the threads at the ends show instructive results.)

EXPERIMENT 12. A correlative experiment to the above, to test the effect of removing the pressure from a turgid and rigid structure, is needed. It may be done thus: Take a young internode of a twining plant or its equivalent (thus, roots of Lupinus albus or beans grown in sphagnum) and place in a 10% salt solution (which causes an exosmotic movement) for a short time, and observe effect. It is well to measure the length of the internode before and after this treatment, and also to determine by use of weights the amount of force required after the treatment to make the piece its former length. Or a delicate leaf may be placed in the salt solution, with an instructive result.

Here the student should make some microscopical observations upon plasmolysis, using Tradescantia or Nitella, with salt or sugar solutions of different strengths, from 2% upwards. Very exact quantitative determinations of the turgor-pressure in cells may be made by use of equimolecular solutions of potassic nitrate, directions for which may be found in Detmer-Moor, 151. As material, rings from dandelion scapes or pieces of Ricinus hypocotyl are recommended.

It is doubtful if the measurement of the force necessary to pull a plasmolyzed tissue back to its length before plasmolysis has much meaning as an index of the turgor-pressure in the cells.

Since the absorbing systems of roots consist of closed membranes, it is obvious that minerals cannot pass into them in the solid form. The only alternative possibility is that they may be absorbed dissolved in the water.

27. Can plants absorb minerals in solution in water through the roots?

Answer by Experiment 13.

EXPERIMENT 13. This may be tested by comparing the growth of seedlings in water containing minerals, with the growth of those in water lacking them. Prepare two germinators as follows: Take two common tumblers with sloping sides, and make rings of wire or glass tubing of such a size that they may be supported by wire or tubing inside and about half-way up the tumblers. Sew tightly over these rings pieces of cheese-cloth which hang to the bottom of the tumblers. Fill one to near the ring with distilled water, and another to the same height with nutrient solution. Sow about twenty radish or mustard seeds on each cloth, and observe the comparative rate of growth. Place black paper around the bottoms of the tumblers to keep the roots in their natural darkness and to prevent the development of Algæ in the nutrient solution (see Fig. 13).

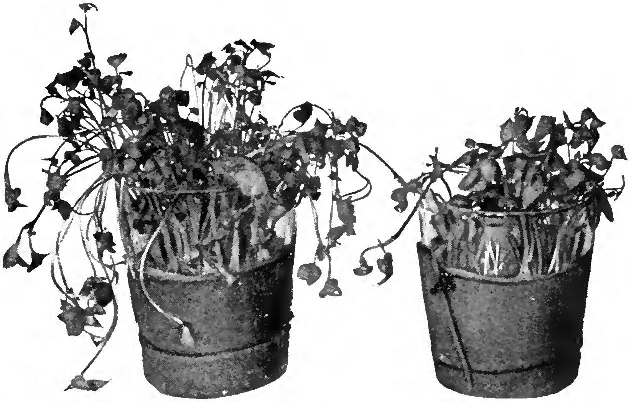

FIG. 13.—SIMPLE WATER-CULTURE VESSELS.
One-third the true size.

(Cheese-cloth sometimes does not absorb water readily, in which case it may be helped by thorough boiling; or instead of it, thin sheets of cotton batting may be used to advantage.)

A better method of supporting the cheese-cloth is to cut the inside from corks, making floating rings a little smaller than the diameter of the tumbler; or, still better are rings of an inch in depth turned from pine on a lathe.

The nutrient solution is to be made up from one of the formulæ commonly given in physiological works, as for instance Detmer, 2, 3; Goodale, 251. It is best to make up a stock for use of the class, but it should be made freshly each year.

A more direct proof that a mineral salt may be absorbed in solution through roots would probably be found by growing seedlings in a dilute solution of lithium citrate, and subsequently testing with the spectroscope the bands given by holding sections of the tissue in the bunsen flame. But perhaps such absorption is too obvious to need special experiment.

A. *Absorption (b) of Gases.*

28. What is the structure of a typical absorbing system for gases?

Answer by a study of a typical leaf.

Construct a diagram of a leaf as a gas-absorbing apparatus, bringing out the path of the gas from the outside world to the cells where it is to be used.

For a "typical" leaf, one of those of a mesophytic deciduous tree or shrub, such as Syringa or Maple, would be best. Much easier to obtain in winter and to section and examine is Ficus elastica, which while not "typical" is sufficiently so from the present point of view. Owing both to the firmness and the high degree of differentiation of its tissues, this leaf is an excellent one for studies on leaf anatomy, particularly for beginners.

Observation of the anatomy of leaf or stem shows a system of air-spaces communicating with the air outside through stomata. To be efficient, all air-spaces should thus communicate with stomata, or their equivalent lenticels.

29. Are the intercellular spaces through long distances in the plant continuous with stomata?

Answer by Experiment 14.

EXPERIMENT 14. This may be tested by forcing air into one part of a plant and noting whether it will issue from stomata at a distance. Select a leaf with its full petiole of a Rubber-plant (Ficus elastica); slip a small stout rubber tube, a foot long, over the end of the petiole and tie it firmly; to the other end of the tube fasten a bicycle-pump; place the leaf-blade under water (to render visible any air issuing from the leaf), pump air vigorously into the petiole, and observe result.

Leaves of Calla (Richardia) are very good for this, and the floating leaves of water-lilies, etc., are especially effective; but most leaves readily give the result.

A gas indispensable to the work of the leaf or other green parts is carbon dioxide. The question arises as to whether this is absorbed mainly or entirely through the stomata, or whether it may pass through the epidermis itself. It has been settled, as will later be referred to (page 90), that it passes through the stomata into the leaf. The study of the living tissues of the leaf shows that the walls of the individual cells are imperforate, and hence gases cannot pass through them directly. As the living cell-walls are always moist, the question arises:

30. Can a gas essential to the work of the living green cell, i.e., carbon dioxide, pass through a moist membrane?

Answer by Experiment 15.

EXPERIMENT 15. This may be tested by placing the gas in contact with a wet membrane having on the other side a liquid which gives a visible reaction with it. Fill a wide-mouthed vial with filtered lime-water, and cover with soaked parchment paper tied tightly on the mouth. Fill a large test-tube with a mixture of 90% air and about 10% CO_2 (by the usual method over water by aid of the CO_2 generator, see page 42), slip the vial into it, and cork up tightly. If the CO_2 can pass through the membrane, the lime-water will become milky in a short time.

(*The membrane must be kept moist by the lime-water; if the vial is not entirely filled by the liquid, the whole apparatus may be kept tipped somewhat; a control experiment in which the CO_2 is removed from the air of a test-tube by potash solution is valuable but not necessary.*)

31. What is the nature of the absorption of substances other than the water, minerals, and gases already considered, i.e.,

By Insectivorous Plants?
By haustoria of Parasites?
By Mycorhiza?
By tubercles of Leguminosæ?

Experiment upon this subject is hardly practicable here. The subject must be studied theoretically from reading, lectures, etc.

32. What ecological effects can you trace from the interaction of the physiological conditions of water, mineral, and gas absorption, and of osmotic phenomena in plants, with the conditions of the external world?

CORRELATED TOPICS.

Theories of osmotic pressure. Plasmolysis. Isotonic coefficients. The micellar hypothesis of the structure of membranes.

Effects of temperature upon osmosis in roots, and the very important ecological consequences.

Mechanical value and use of turgescence. Tension of tissues, and rigidity.

Distribution and movement of air, minerals, and water in the soil, and the resultant upon the structure, size, and form of the root, and other parts. Mechanical analysis of soils.

Physics of entrance of gases through stomata. Positive and negative gas-pressure in plants.

Physics of transfer from root-hairs to ducts.

Absorption of water by green parts, and special structures; and by seeds.

Water-culture.

Corrosive power of roots; their "selective power."

LITERATURE.

On Osmosis there are important notes in Nature, vols. **54**, **55**, and **58** (see index to those volumes).

Note by Leavitt in American Journal of Science, **7**, 1899, 381, first part only.

Particularly see Brown, Vice-Presidential address in Nature, **60**, 474, 544.

B. *Transfer (a) of Water and Minerals.*

You have found that the water absorbed by root-hairs is transferred to the ducts and given a good start up the stem. It is now to be traced through the plant.

33. What is the exact path of transfer of water through the stems of plants?

Answer by Experiment 16.

EXPERIMENT 16. This may be tested by coloring the moving water through use of a harmless dye in stems transparent enough to show the presence of the color. As the dye will not enter living roots, cut shoots must be used. Cut under water (see page 44) a shoot of the translucent Impatiens Sultani or similar plant, and transfer it, without exposure to air, to a shallow dish containing water deeply stained with eosine or safranin; clamp it with its end just dipping into the liquid and observe results. The exact path may be determined by examining longitudinal and cross slices with a lens.

Watching the ascent, what do you think is the power at work?

34. What is an average rate of ascent for water?

Answer by observation of Experiment 16. Is the rate of ascent of the dye probably the same as the natural ascent of sap in the uninjured plant?

The usual method of testing this is by use of Lithium salts recognized spectroscopically (see Detmer-Moor, 233). This test is said to be not difficult to apply.

35. What is the anatomical structure of the water-conducting tissues of a plant?

Answer by studies on the stems of such typical plants as Aristolochia and Zea (or by review of earlier studies). Construct a diagram of the stem as a sap-conducting apparatus.

In the examination of the stem carrying dyed water, you find that walls as well as cavities contain the color, which suggests the possibility of water-passage in the walls.

36. Can cell-walls transfer water?

Answer by Experiment 17.

EXPERIMENT 17. This may be tested by supplying water to one part of a mass of tissue composed of dead cell-walls only and noting whether it spreads. Select a piece of dry wood a few cm. square and 3–5 mm. thick; allow a strip of filter-paper, with one end in a water-reservoir, to rest against the middle of its under side, and carefully note the result, explaining it in terms of micellar processes, and diagramming the principal stages in the result.

Does the effect upon the wood suggest a merely passive absorption of the water, or a more energetic process?

This may be answered by noting how much power is needed to force a warped piece of the wood back into its former shape, though this will not give a measure of the molecular force involved in the imbibition.

The use of absorption of water by dry tissues to produce movements is important and widespread in plants.

37. What is the nature of the absorption in hygroscopic awns?

Answer by Experiment 18.

EXPERIMENT 18. Mount a hygroscopic awn of Erodium or Stipa by fixing it vertically on a large cork with sealing-wax; if the upper end does not possess a horizontal projection usable as a pointer, add one with sealing-wax. Place successively in wet and dry chambers and observe movements of the pointer. Explain the results upon a micellar basis. (Place it also in a steam-jet.)

A telling demonstration of the power with which dry tissues absorb water is given by placing dried peas (Soja beans are particularly effective) in a narrow-necked bottle, until it is full, when it is to be filled also with water and immersed in water. The process is, however, here complicated by osmotic phenomena.

The mechanics of the twisting of hygroscopic awns is discussed by Murbach in Botanical Gazette, **30**, 113. The great value of Soja beans for imbibition experiments is pointed out by Copeland in the same journal, **29**, 347.

38. What is the power which produces the ascent of sap?

No experiment upon this subject is practicable. Although obviously of very great importance, it is still unsettled. A full theoretical treatment of it should be worked out and a concise account given.

One phase of it which may easily be studied experimentally is the influence of root-pressure in aiding the ascent in small herbaceous plants. This may readily be investigated as follows: Root-pressure is to be removed by cutting off the shoot and placing it in water; then, after it begins to wilt, the pressure is to be restored by placing the shoot in water in one arm of a long U-tube and pouring mercury into the other arm. (See MacDougal, Physiology, 32.) The mercury tube used for testing joints (see page 41) is well adapted for this purpose.

A number of practicable experiments upon this subject are given by Darwin and Acton, 88–96. The experiments given in some works to illustrate the lifting power of evaporation seem to me to have no bearing upon this question, since the physical conditions, particularly the operation of atmospheric pressure upon the exposed liquid, are so very different in the two cases.

B. *Transfer (b) of Elaborated Substances.*

39. What is the path and the physics of the transfer of elaborated food-substances?

No experiment is here practicable upon this subject. It is to be worked up theoretically, and expressed in a concise paragraph. (Note Detmer-Moor, 351–359.) Construct a diagram showing the structure of this system.

The well-known constriction-experiment, proving the descent of food-materials in the bark, is easy of trial in summer.

CORRELATED TOPICS.

The theories of sap ascent.
Use of ferments.
Ecological uses of hygroscopic absorption and transfer of water.
The latex system.

LITERATURE.

Ward, H. M. Timber and some of its Diseases. London, 1893. (Chapter IV.)
Darwin, F., and others. Discussion on Ascent of Sap. Annals of Botany, 1896.
Review of Strasburger's "Leitungsbahnen" in Annals of Botany, 1892, 227.
Noll, F., in the Bonn Text-book.

C. *Transpiration.*

Your earlier experiments have proven that large quantities of water are absorbed by roots and passed up stems. The question next arises, what becomes of it? Is any of it given off into the air from the leaves, and if so, how much?

40. Is water given off by the leaves of a common plant growing under normal conditions, and if so, in what amount?

Answer by Experiment 19.

EXPERIMENT 19. To test this, a method must be used by which loss of water from leaves of an uninjured plant may be exactly calculated. For this a potted plant is convenient, but, obviously, evaporation from the soil and pot must be prevented. Take a Ricinus or other leafy plant, and a glass jar just large enough to hold the pot; cut a small hole in the center of a piece of rubber cloth which is somewhat larger than needful to cover the top of the jar; slip the rubber up over the pot and tie to the stem by a stretched rubber band, and fasten the cloth over the top of the jar by a tight wire (see Fig. 14, plant in the center.) Insert a short thistle-tube through a tiny hole. Make sure that the pot is enclosed water-tight. Weigh on the spring-balance three times daily, as near sunrise as possible, at midday, and at sunset, and water once daily through the thistle-tube with an amount about equal to that given off. Keep a record of temperature, moisture, and sunshine, and plot all four records together.

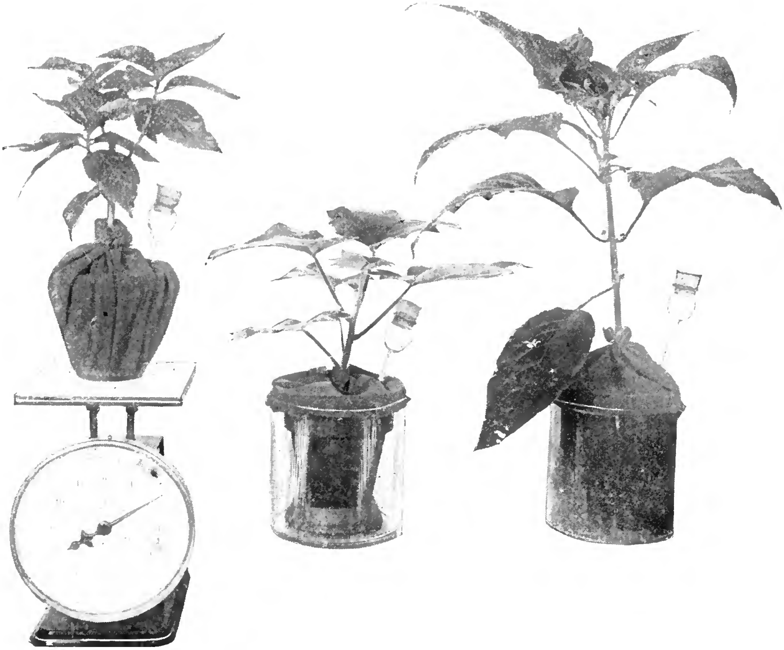

FIG. 14.—PREPARATION OF PLANTS FOR STUDY OF TRANSPIRATION. One-fourth the true size.

(In using the spring-balance, the weight must always be placed exactly in the center.)

Another method of preventing evaporation from the pot is to wrap it completely in rubber cloth, gathered up and tied about the stem of the plant as shown in Fig. 14 on the left. The use of the glass, however, has three advantages: it allows the condition of the soil to be seen, thus giving guidance to the watering, it allows a somewhat freer access of oxygen to the roots, and it makes possible the experiments described under Experiment 21. Another method which has advantages is shown in Fig. 14 on the right, where the plant is removed from the pot and placed in a battery-jar with enough extra earth to fill the latter; the jar is then covered with the rubber precisely as described in the above experiment. In this case the plant should be allowed to stand for a day or two before using, in order that it may become accustomed to the new conditions. (See Note 4 of the Addenda.)

Another method, with manifest advantages, of preparing the plant is given by Barnes (Plant-life, 394) as follows: "Clean and dry the surface of a pot in which a thrifty single-stemmed plant is growing; close the hole in the bottom with a cork; with a brush paint the whole surface with a thick layer of melted paraffin. Cut out a piece of stiff paper which will fit around stem and just cover the soil in pot. Using this as a pattern, cut a cover for the soil from a sheet of lead; slit the cover from the central hole to circumference, adjust it around plant, and cement all cracks with grafting-wax." Or the pot can be set in a tin vessel which it fits and the lead cover luted to this. Professor Barnes adds (in a letter): a disk of filter-paper should be added to the bottom of the paraffined pot to prevent adhesion to the table, etc.

A spring-balance for the weighings is much more convenient (particularly in saving time) than a balance using weights, but, even if of the best kind, is accurate enough only for very rough work (see page 33). An ordinary balance can be used and should be sensitive to 1 gr.; the torsion form is excellent. For very exact work a more accurate balance is needed, and one especially fitted for the purpose is described by Hansen in Flora, **84**, 355. Very well adapted to this work is the 3423 balance of Gerhardt (see page 33). An autographic transpiration machine is described by Copeland in the Botanical Gazette, **26**, 343, and another on the general principle of Copeland's, but taking a potted plant, by Corbett in the Twelfth Annual Report of the West Virginia Experiment Station. The objection to machines on the principle of these two is the difficulty of keeping the water in the reservoirs at a constant temperature, as must be done to secure accurate results. A very exact form of autographic transpiration-recorder is described by Woods in the Botanical Gazette, **20**, 473, and a registering balance by Anderson in Minnesota Botanical Studies, No. **16**.

I have made attempts, with but indifferent success, to secure an autographic transpirometer by keeping the plant on the spring-balance, and running a thread from the top of the latter over the multiplying-wheel brought into action with the recording cylinder (see later, page 102). This would work if the spring-balance were delicate enough.

For taking temperatures a thermograph (page 33) is best. For moisture, a hygrograph (page 33) is best, though a wet and dry bulb apparatus frequently observed, will do. There are several forms of sunshine-recorder, but for most purposes an approximate record traced by some student for the class upon a thermograph sheet kept beside him for the purpose is sufficient. All of these records should be plotted upon the sheet with the Transpiration curve.

There are several other methods of investigating transpiration, of which one of the most practicable is the measuring method described by Detmer (page 217). A very simple and ingenious method of demonstrating transpiration and intercellular aeration together is given by Noll in Flora, **86**, 386. Very useful for some purposes are potometers, several forms of which are described by Darwin and Acton. A very practicable form is described by MacDougal in the Botanical Gazette, **24**, 110. A modification of this, which I have found very efficient, is shown in Fig. 15. A T-tube is bent into the form shown by the figure and attached by a water-tight joint to a large-bore thermometer-tube, backed by a millimeter scale. The plant, carefully cut off under water, is fixed by a water-tight joint in one arm of the T-tube, great care being necessary to prevent compressing the ducts while the joint is being made. To the other arm is attached a tube with a glass stopcock, which, however, may be replaced by the simpler arrangement of rubber tubing and compressor shown at the bottom of the figure. The

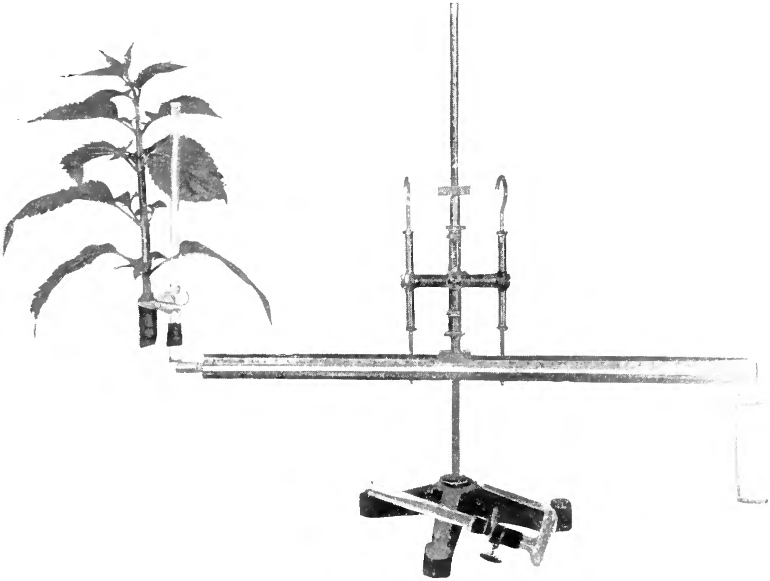

FIG. 15.—A SIMPLE POTOMETER. One-third the true size.

whole apparatus, including the vial at the right-hand end, is filled with water (boiled to free it from air), and as the plant transpires, a bubble of air, allowed to enter the tube through the vial end, moves along the capillary tube. Its rate may be exactly determined by use of the scale and metronome. When it has nearly reached the end of the tube, the stop cock is opened and the weight of the water in the vertical tube forces the bubble back to the other end of the capillary tube. This apparatus is particularly adapted to studies upon the influence of different conditions upon transpiration, since it may so readily be moved about and the plant can be placed in closed receivers, etc. The method assumes that the rate of transpiration and absorption are equal, which may not always be the case, and with me the potometers never work very evenly.* In using it, it is better to employ some stiff-leaved plant, as more delicate ones are apt to wilt. Plants with their own roots, raised by water-culture, should give good results. As arranged above, only relative rates of transpiration are determined, but these may be made definite by use of a small tube of known diameter in place of the capillary tube. (See Note 5 of the Addenda.)

41. How is the rate of Transpiration affected by external atmospheric conditions, i.e., by variations in heat, light, moisture?

Answer by Experiment 20.

EXPERIMENT 20. To test this, a plant arranged as for Experiment 19 may be used, and the conditions varied thus (or in some better way invented by yourselves): more moisture by putting a large bell-jar over the plant; less light by a dark screen (use only in sunlight); lower temperature by opening the ventilators. The plant should be kept for at least two hours under the new condition, after obtaining for four hours a record for the normal. Every precaution should be used to change only one condition.

Although this line of work is of very great physiological and ecological importance, it is very difficult to carry it out satisfactorily with simple appliances. It is difficult to keep the external conditions constant for a length of time great enough to allow of good records by weighings. Here potometers, despite their shortcomings, afford fair results, as they give ample records within a few minutes.

Observation shows that in nature it is often the case that the temperature of the air and of the soil to which a plant is

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* So irregularly does the potometer sometimes work that one concludes the fault must be in it and not in the plant. I have made, however, a very careful series of measurements with the instrument shown in Fig. 15, using instead of a plant one of the diffusion-shells (of Experiment 6) filled with water and exposed to evaporation under various conditions. Thus used, the apparatus worked with the greatest evenness, showing that the fluctuations are in the plant and not in the potometer.

exposed are very different. The effect of air temperature upon transpiration has been found in Experiment 20; we have next to ascertain—

42. How is the rate of transpiration affected by changes in soil-temperature?

Answer by Experiment 21.

Experiment 21. To test this, the soil temperature of a plant must be varied independently of that of the top, which may be done by placing the roots of a growing plant in a glass jar, the temperature of which can be raised and lowered while radiation from it to the shoot is cut off by a woollen covering between. Prepare a plant as shown in Fig. 14 (on the right). Thrust a thermometer through the rubber into the soil, and hang another in the air by the shoot on a stick thrust through the rubber. Weigh; then cool the glass jar to about 5° by immersing it in water containing melting ice or snow; keep it at 5° three hours, then remove the jar, dry it, and weigh. Later warm the jar by slowly warming the water with a spirit-lamp until it reaches 38° to 40°; keep it there for three hours, and weigh. In both cases cover the jar with a woollen wrap and set the whole in an ordinarily favorable situation.

The importance of this experiment consists in the light it throws upon the ecological significance of the occurrence of xerophytic characters in many hydrophytic plants.

43. What is the construction of the Leaf as a transpiring structure?

Answer by a review of your knowledge of leaf-structure, especially including the stomata. Construct a simple diagram of the leaf as a transpiring structure, bringing out the path of the water from the ducts to the air outside of the leaf. Explain by diagrams the working of the stomata.

44. Are stomata indispensable to transpiration in the higher land plants?

Answer by Experiment 22.

Experiment 22. This may be answered by observation of the relative amount of transpiration from the two sides of a leaf in which one side has stomata and the other none. Select such a plant, e.g., Ficus elastica; place upon the two sides watch-crystals held in place by a spring or similar device. Set the leaf vertically so that both sides may receive about equal illumination, and observe the relative transpiration as measured by the water collecting inside the glasses. (*The glasses may be sealed to the leaf by use of soft wax, page* 40, *but it is not necessary.*)

Another very excellent way of testing this is through use of Stahl's cobalt chloride method. If slips of filter-paper be dipped in a 4 to 5% solution of cobalt chloride and then dried over a flame, they will be blue in color, but will turn red on access of any moisture, the more quickly the greater the moisture. Pieces of the paper put in the two watch-crystals used above give good results, or they may be simply placed against the leaf surfaces and covered from the moisture of the air with bits of mica, held in place by clamps. The method is used by Stahl to determine whether stomata in given leaves are open or closed. There are other excellent methods of testing this by use of calcium chloride, an eager absorbent of water, which is weighed (Detmer, page 215); by use of hygroscopic awns (Darwin and Acton, 103); and by other methods described in the works on the subject. (See Note 6 of the Addenda.)

45. Is water ever given off by the aerial parts of plants in other form than vapor?

Answer by Experiment 23.

Experiment 23. Of course the liquid form is the only other ordinarily possible. To test whether liquid water is thus given off, a vigorous young plant of high transpiring power should be selected when in full transpiration (and conduction), and the transpiration checked. This may be done in various ways, but most conveniently by darkening the plant and surrounding it with a saturated atmosphere. Select young plants of Tropæolum, grasses (preferably seedlings), corn, or others, cover with a bell-jar and place for one or two hours in a warm dark place.

How may the result be interpreted?

46. Construct a diagram (based upon all of your sources of information) showing in the simplest possible way the path of the water from the soil to the air through the plant. Indicate the known and supposed physical forces at work at the different points.

47. What ecological results and aspects of Transpiration can you think of?

Answer from your own observation, and from other available sources.

48. Prepare a synoptical essay, of not over 500 words, upon Absorption, Transfer, and Transpiration in Plants.

CORRELATED TOPICS.

Theories as to the fundamental significance of the copiousness of transpiration.

Ecological aspects of Transpiration and its relation to plant form and position.

Causes of wilting; relation of checking of transpiration to restoration of turgidity.

Ice-formation on plants.

Hydathodes.

Summary of the various offices of water in the Plant.

LITERATURE.

Brown, H. T. Vice-Presidential address in Nature, **60**, 474.

Stahl's and other important works should be traced through Pfeffer.

D. *Metabolism, (a) Photosynthesis.*

You have learned from studies in earlier courses that plants make starch in their green parts in the presence of light, a process called Photosynthesis. We have now to examine this process in some detail.

49. What is the physical and chemical composition of the usual end-product of Photosynthesis, i.e., Starch?

Answer as to chemistry from your various sources of information; as to physical composition, by an observational and polariscopic examination of starch grains from the potato (scraped from a cut surface, and mounted in water).

Apply and observe the effect of the iodine test (described in 51).

50. What structures are concerned in Photosynthesis?

Answer by diagrams showing:

(*a*) Quantitatively and qualitatively the distribution of Chlorophyll in a complete higher plant, and

(*b*) The structure of the most specialized green chlorophyll-bearing organ, and

(*c*) The structure of a single green cell.

51. Is light absolutely essential to Photosynthesis?

Answer by Experiment 24.

Experiment 24. This may be tested by placing similar leaves for a time under conditions precisely alike except that one is exposed to light and the other kept in darkness, and then applying a test for starch. Place a Tropæolum or other convenient thin-leaved plant for two nights and a day in darkness (to empty it of starch); as early as convenient the next day bring it into light; cover one leaf completely on both sides with tin-foil, leaving another beside it uncovered; *or*, cover a leaf with tin-foil in which, matching above and below, some pattern has been cut (see Fig. 16), leaving the leaf there exposed to light on both faces; *or*, by means of pins thrust through the leaf, place two pieces of cork on opposite sides of a leaf, matching above and below (see Fig. 16). Keep the plant all day in bright sunlight, and towards sunset place the leaves for a few minutes in boiling water (to kill them and swell the starch), and place in 70% alcohol. Later, at a convenient time, warm the alcohol in a water-bath and renew it until all the green is removed, and the leaves are blanched. To bring out the starch distribution, place them in a

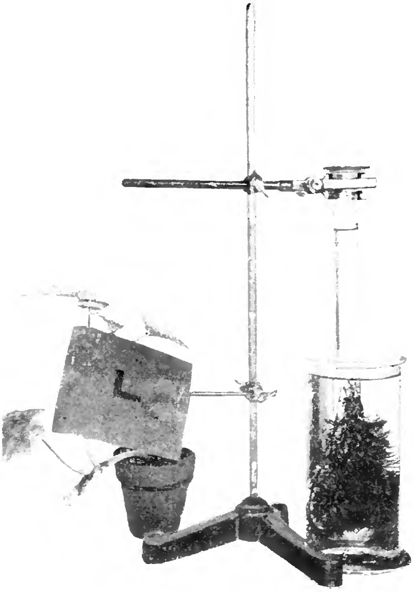

Fig. 16.—Arrangements for Study of Photosynthesis. (Instead of the black paper cover on the left, tin-foil should be used.) One-third the true size.

porcelain or other white-bottomed dish, and pour over them an iodine solution (made by dissolving solid iodine in weak aqueous solution of potassic iodide until the mixture has a dark wine color), which will turn all starch dark blue.

(*In warming the leaf in alcohol to blanch it more rapidly, the alcohol should be placed in a porcelain dish in a water-bath; and the flame must not be allowed to reach it.*)

For the great majority of plants, perhaps for all, the preliminary day or more in darkness is indispensable to the success of the experiment. When tried out of doors it is not enough to simply enclose the leaves in a black bag or box unless this is also shaded, as the heating in sunlight injures the parts. Doubtless the best way would be to run the branch into a double-walled dark box. Particularly good leaves for the experiment are cucumber and Abutilon. Thick leaves like those of Ficus are not so good, since it takes long to empty them of starch.

Of much value is experimentation to prove the increase in dry weight through Photosynthesis. There is, however, no easy method of accomplishing it, and it hardly seems profitable to take the time necessary for the very exact weighings, though these have considerable educative value. Such weighings, moreover, have only a qualitative value, since the loss by respiration cannot be determined. The best known method, that of Sachs, is described in Darwin and Acton, 31; another method is given by Detmer, 9. In place of the corn plant used by him I have used with some success Chinese lily-bulbs grown in water, though the possibility of error is here greater than in the seed method.

52. Is amount of Photosynthesis proportional to amount of sunlight?

Answer by an experiment (to be called Experiment 25) invented by yourself.

(*Remember the golden rule of Experiment, i.e., change only a single condition.*)

The very important question now arises, why is Chlorophyll green? Since its work is dependent upon light, and since sunlight includes many rays of different properties, it would seem probable that the chlorophyll color must be connected with some peculiarity of the nature of light in correlation with the nature of the chlorophyll work. To test this, we must first find out—

53. What effect is produced upon sunlight by its passage through chlorophyll?

EXPERIMENT 26. This may be settled by examining the effect produced upon a ray of light by its passage through chlorophyll in comparison with one not passed through the chlorophyll. This examination must be made with an instrument designed for light analysis, namely, the spectroscope (see page 33), while the chlorophyll is best examined in solution. Prepare a solution of chlorophyll from clear green leaves as yielded by Experiment 24; it should not be over a few hours old, and, to prevent its alteration in light, must be kept in darkness while not in use. Place the solution in three flat-sided Soyka flasks, which may be supported one, two, or three at a time before the spectroscope (see the arrangement in Fig. 17); examine these, one, two, and three at a time, with the spectroscope, and compare the solar spectrum pure and as affected by the chlorophyll. Try to interpret the result, and record the spectra as nearly natural as possible.

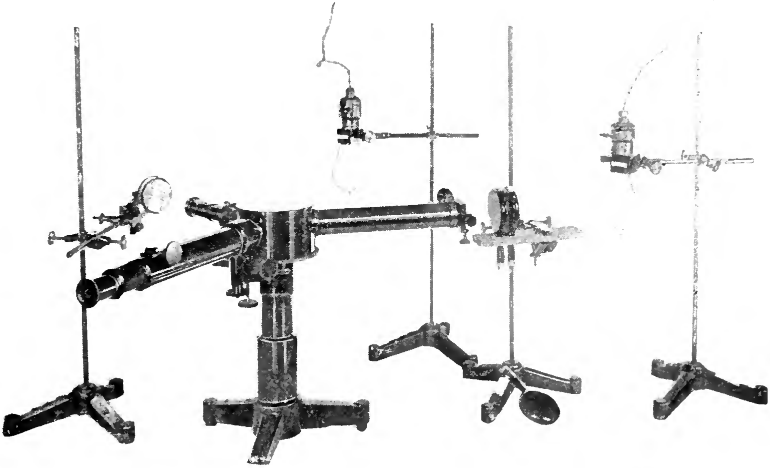

FIG. 17.—ARRANGEMENT OF SPECTROSCOPE FOR STUDY OF ABSORPTION SPECTRUM OF CHLOROPHYLL.

The light in the middle supplies both the scale and the comparison prism. One-fourth the true size.

Practically it is just as instructive, and certainly it is much more convenient, to use instead of the sunlight the incandescent electric light. A good arrangement is shown by Fig. 17. A comparison should of course be made between electric light and sunlight, but the principal study may then be carried on with the former.

Incidentally the other leading characteristics of chlorophyll should be noted, particularly its fluorescence, which may be heightened by focusing sunlight upon it with a hand-lens.

Instead of the ordinary spectroscope, a microspectroscope may be used as described by Detmer, 22. Particularly good colored plates of chlorophyll spectra are given in the Frank and Tschirch diagrams, Nos. XV, XVI.

The observation of the character of light passing through living leaves is easily practicable and instructive. Various thicknesses of leaves may be placed between the source of light and the spectroscope until the light just disappears. Also an instructive result is given by observing this light by the eye directly. For this should be provided a tube about 20 to 30 cm. long and 2 to 3 cm. diameter, closed at one end and provided with a cap just fitting the closed end. It may be of pasteboard. Holes half the diameter of the tube should be cut in end and cap. Circles of fresh leaves nearly the diameter of the tube may then be placed between end and cap; then the tube, with the eye at the other end, should be pointed at the sun. No light should leak into the tube, which should be blackened throughout. Successive layers of leaf should be added until finally no light passes through, and the color of the last vanishing rays should be determined.

The preceding experiment shows that chlorophyll absorbs certain rays from light, allowing others to pass through it. This suggests that the absorbed rays may be the ones used in the process of Photosynthesis.

54. Are those light-rays particularly absorbed by Chlorophyll the ones which are active in Photosynthesis?

Answer by Experiment 27.

EXPERIMENT 27. This may be tested by allowing the principal rays absorbed by Chlorophyll, i.e., red and blue, to act upon a leaf, and noting the amount of starch formation in comparison with that made under rays not absorbed by Chlorophyll, i.e., green. These rays may most conveniently be applied by use of colored liquids, each of which cuts out all rays except its own color (which can be determined only by use of the spectroscope). The leaves should be prepared for the experiment and the test for starch applied as in Experiment 24. The colored liquids may be made thus: for red, the dye "scarlet"; for blue, ammoniacal sulphate of copper; for green, a carefully tested mixture of potassium chromate and ammoniacal sulphate of copper. These should be placed in small square bottles which are held by clamps upon the two surfaces of the leaf so that the colors match on the two surfaces; the leaves thus prepared should be exposed to bright but not intense light for a day, and in the evening tested for their starch contents.

(*In preparing the solutions, the spectroscope must be used to deter-*

termine that they are made of just such a density that the full bottle transmits its own color only, and as much of that as possible.)

Colored glass would seem available for this purpose, but the spectroscope shows that none of the colors obtainable are pure; the eye is an entirely unsafe guide to the quality of colors, as it does not resolve mixtures. Probably the liquids could be arranged in some better way than the above, which gives only fair results. Gelatine plates are better than glass, for the colors are obtainable nearly pure for red and green, though the blue is poor. (The gelatine known as No. 073 gives a perfect red, while 052 gives a fair blue. No good green is available.) Attempts made by my students to dye sheets of colorless gelatine by use of the above-mentioned colors have not been successful. The ideal method of applying colors to a leaf for this purpose would be one in which both red and blue light are supplied together to the same piece of leaf, which obviously cannot be done by solutions containing both colors, nor by use of two colors of gelatine sheets placed together. (See Note 7 of Addenda.)

Another method of testing the effect of colored rays and other external conditions upon photosynthesis is that of counting the oxygen bubbles given off by water-plants placed under the special conditions (see Darwin and Acton, 36), either in the spectrum itself, or behind colored screens. But it is hardly practicable here. On the use of pure-color screens, consult the paper by Pennington, in Contributions from the Botanical Laboratory of the University of Pennsylvania, **1**, 203.

Also, for this purpose the pure-color chamber later described (page 108) should be available, by the use of small seedlings grown low in small pots, but I have not yet thoroughly tested it in this way.

You have learned (Section 49) that starch contains carbon. The only known source of supply of this element for most plants is the CO_2 of the atmosphere, though small quantities may be absorbed by some plants in compounds from the soil.

55. Is atmospheric CO_2 necessary for Photosynthesis?

Answer by Experiment 28.

EXPERIMENT 28. This may be settled by determining whether starch-formation can go on in an atmosphere deprived of CO_2. Prepare two large bottles with wide mouths, and rubber stoppers through which chloride of calcium tubes have been fitted (Fig. 18). Cut under water two small shoots, holding two or three leaves each, from a plant prepared as for Experiment 24, and place these in vials nearly full of water. Place shoots and vials in the bottles, and on the bottom of one put a quarter-inch depth of soda lime (a powerful CO_2 absorber) and fill the tube of the same with the soda lime. In the other (the control experiment) place fine sawdust in place of the soda lime, to keep the physical conditions as nearly as practicable the same. Expose to bright light for a day, and test for starch.

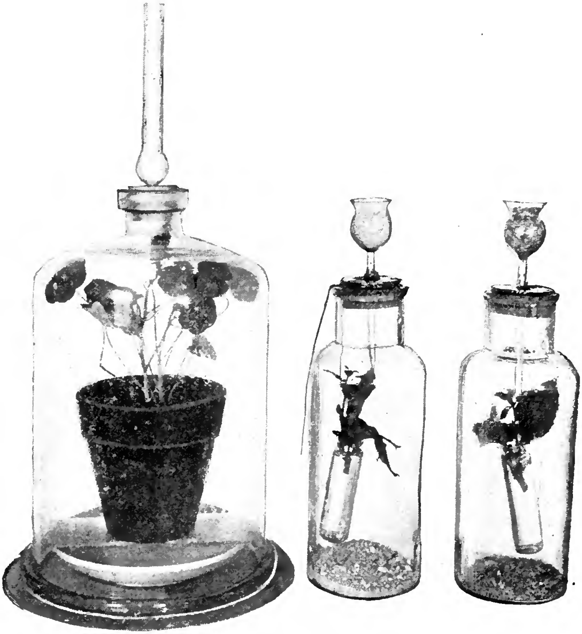

FIG. 18.—ARRANGEMENTS FOR DEPRIVING LEAVES OF CARBON DIOXIDE. One-fourth the true size.

This experiment may also be tried, and for some reasons more advantageously, by using entire potted plants placed in bell-jars sealed by vaseline to ground-glass plates (Darwin and Acton, 29; also Fig. 18, left). Through rubber stoppers should pass calcium chloride tubes containing soda lime and sawdust respectively, and a dish of soda lime should be placed in one bell-jar. Otherwise the experiment proceeds as above.

It is also possible to test easily whether CO_2 enters the leaves through the stomata or the epidermis. If a thin leaf containing stomata upon one surface only (such as Primula) be selected and the plant kept in darkness for a day, and the surface containing the stomata be coated with vaseline, no starch is made as shown by the usual test, while a neighboring leaf coated with vaseline on the upper surface makes it abundantly. Or, of the two halves of the under surface, one may be coated and the other left free, with the same result.

The need for carbon dioxide, and hence its probable absorption, in photosynthesis, shown by Experiment 28, suggests, in view of the well-known reciprocal exchange of carbon dioxide and oxygen in other physiological processes (i.e., animal respiration), that oxygen may be released in Photosynthesis.

56. Is the absorption of Carbon dioxide in Photosynthesis accompanied by a release of Oxygen?

Answer by Experiment 29.

EXPERIMENT 29. This may be tested by supplying to a plant under conditions favorable for Photosynthesis a measurable quantity of CO_2, and after a time testing whether it has been replaced by O; this may be very conveniently effected by burning a candle in a closed chamber, which, it is known, absorbs O and gives off CO_2; when it has given off about 3% of CO_2 it goes out and can only burn again after the O has been restored. Early on a bright day prepare three large wide-mouthed bottles and three ordinary saucers, with a pneumatic trough or other large vessel of water (see Fig. 19). On a flat cork smaller than the inside of the neck of the bottles place a small candle, and attach by a tack a string to the middle of the under side of the cork. Float the cork on the vessel of water with the candle lighted, and hold over it, with the mouth dipping into the water, one of the bottles. When the candle goes out withdraw it by the string and treat the other two bottles similarly

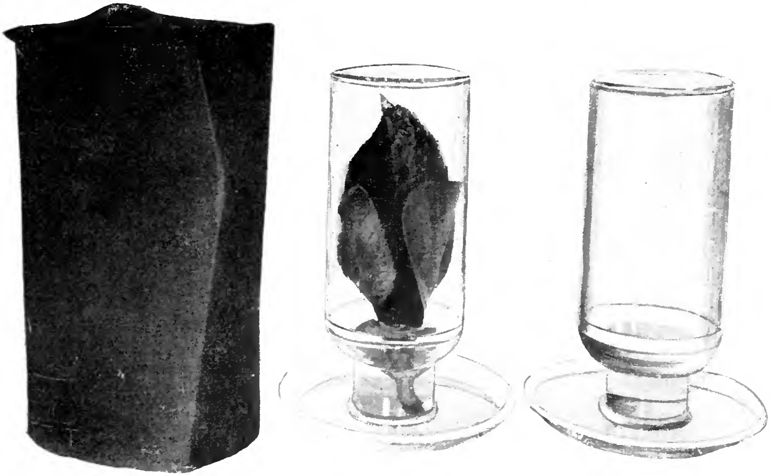

FIG. 19.—SIMPLE ARRANGEMENT FOR TESTING THE GAS-EXCHANGE IN PHOTOSYNTHESIS. One-third the true size.

Then into two of the bottles insert under water large vigorous shoots of a green plant, slip the saucers beneath them and remove from the large vessel; put one of them in the bright sunlight, and the other beside it but covered by a dark hood from the light (Fig. 19), and let the empty bottle also stand as a control in the light. At evening withdraw the plants by aid of the vessel of water; lift one bottle after the other cautiously from the water and replace it quickly over the floating burning candle, noting the time the candle will burn in each.

(*If corks just fitting the bottles are available, a better way to make the final test is to cork the bottles under water, invert them, and lower the candles into them, removing the corks as little as possible. It is well to use a shoot with a large quantity of leaves.*)

The experiment may be tried without the saucers and water-vessel by using preserve-jars, the stoppers of which may be screwed on air-tight; lighted candles are lowered into them after the shoots have been inserted. Ordinary corks will not do for this, as they allow some gas diffusion unless thoroughly covered with vaseline.

Another very excellent method often used for testing the release of oxygen in Photosynthesis is described in various books (as Detmer, 37; MacDougal, 38). A quantity of a water-plant such as Elodea (Anacharis) or Cabomba is placed in light in a glass vessel under a funnel over which is a test-tube filled with water. The gas rises, guided by the funnel into the tube, where it may be tested for oxygen by a glowing splinter thrust into it. A rather more convenient way to apply this test is given by Hansen in Flora, **86** (though his principle could be simplified). The test may also be made with phosphorus (see page 42). In order to be able to measure the quantity of oxygen taken up by the phosphorus, it is well to use an inverted cylindrical graduate (as in Fig. 16).

If the ends of the cut stems (in Cabomba at least) are thrust into the test-tube (where they may be held tied lightly to a glass rod), the funnel is needless. When the funnel is used, and indeed without it, the outer vessel should be as large and with as great an air-space as is conveniently possible, to allow of the free absorption of CO_2 from the air, and the funnel should be lifted to allow free communication with the remainder of the vessel. Moreover it is very profitable to blow in CO_2 from a generator from time to time. When the evolution of bubbles has stopped through exhaustion of the gas from the water, it will begin again immediately if the CO_2 be thus added. The gas so collected in the test-tube when carefully tested will usually show not over half oxygen; a trifle of the remainder is CO_2, while the remainder is nitrogen. Under favorable conditions, however, the proportion of oxygen may greatly increase. (See Pfeffer-Ewart, page 331.) The plants of course always work much better when in vigorous growth than when resting during the winter. A self-registering form of this apparatus is described by Copeland, in the Botanical Gazette, **29**, 439, but it seems liable to serious practical errors.

It is sometimes stated in books that oxygen is given off abundantly from leaves of land plants plunged under water, the bubbles collecting upon such leaves being supposed to be oxygen evolved in photosynthesis. In fact those bubbles are practically nothing other than air dissolved in the water, released by the heat of the sun, as is shown by the fact that they will collect as copiously upon a leaf-surface containing no stomata as upon one containing them abundantly, and also they will collect upon the leaves as abundantly if the vessel be set in a warm dark place as in an equally warm light place. A test of such bubbles would give a larger proportion of oxygen than air contains, because oxygen is more soluble in water than nitrogen.

An exact quantitative method of studying the gas-exchange is given by Pfeffer (see Detmer, 41). The principle of this, with which I have had very good results, may be much simplified as follows (see Fig. 20): Take two of the special burettes used earlier in Experiment 6, and close their tops by rubber stoppers cut to such a length that when forced tightly in they just fill the space above the graduation, and for additional security against leakage cover them and the burette end with sealing-wax. In each one place now a long shoot with numerous small leaves (I find Ficus repens grown in most greenhouses particularly available for this), to the lower end of which a very fine wire is attached by means of which it may be withdrawn. The wire is then taken outside the burette and held in place by a rubber band. Each burette is then placed over a small mercury reservoir, a very small stiff rubber tube being first run up inside the burette with the other end outside. The burette is now depressed in the mercury reservoir until the mercury stands at 4 cc. above the zero-mark inside and out, with end of the rubber tube just at the level of the liquid. The tube is now with-

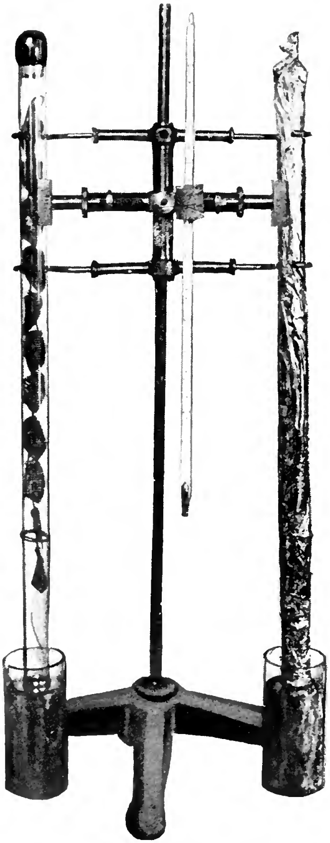

FIG. 20.—ARRANGEMENT FOR DETERMINING THE GAS-EXCHANGE IN PHOTOSYNTHESIS. One-fourth the true size.

drawn and the burette is raised until the zero-mark is at the outside level (it will fall somewhat inside); enough CO_2 must now be allowed to enter the burette through a slender bent glass tube held in the mercury beneath the burette, to depress the mercury to the zero-mark. If properly carried out, the level of the mercury will be exactly the same (and at the zero-mark) inside and out, while the burette will contain 8% of CO_2, an amount which most plants can readily use. One of the burettes is now to be darkened by a covering of tin-foil (better than black paper because it reflects heat), the exact temperature is to be noted from the attached thermometer, and the whole is to be set in bright light for a day. Towards sunset (in winter, a shorter time is ample in summer) the plants are to be carefully withdrawn by the wires and the resultant change in volume, marked by the rise of mercury, carefully noted. The gas analysis can be made at leisure. For this prepare two sticks of caustic potash of less length than the diameter of the burette; attach them to fine wires, and insert them into the burettes; after three or four hours they will have absorbed all the CO_2 in both tubes, when they may be withdrawn. The tubes are now to be depressed until the mercury is at the same level inside and out, and the levels read on the burette graduation. The difference of level in the two tubes will show the total disappearance of the 8% of CO_2 in the tube in light and its presence in the tube in the dark. To test now whether the CO_2 shown to have disappeared from the light tube has been replaced by oxygen, one either transfers the burettes with their reservoirs into a large dish of water (dropping off the reservoirs allows the water to enter the tube) and applies the phosphorus method of removing oxygen, or one transfers them to a dish, such as a large tumbler, containing a concentrated mixture of pyrogallic acid and caustic potash, and drops away the reservoirs as before, allowing the mixture to enter the tubes. As this is an oxygen absorber, the height to which it will rise in the two tubes will demonstrate whether or not the CO_2 of the light tube was replaced by oxygen. The final readings for levels should always be made at the same temperature at which the experiment was started, and theoretically also at the same barometric pressure, but this is hardly practically important. Theoretically it would be better also to make the final readings with the burettes depressed until inside and outside liquid-levels are the same, but this also is hardly practically necessary. Also changes of temperature from local warmth of the hands on the tubes just before readings are made must be avoided. (See Note 8 of the Addenda.)

57. What are the substances, conditions, and processes concerned in Photosynthesis?

Tabulate these fully. Answer from your various sources of information.

D. *Metabolism, (b) Respiration.*[*]

You have found that photosynthesis, the Plant's process of food-making, involves the conversion of working, or kinetic, energy into latent, or potential, energy, with absorption of carbon dioxide and elimination of oxygen. The question now arises as to the conditions of the utilization of this energy. A conspicuous case of its utilization occurs in growth, and hence we may use growth to determine what the gas-exchange in energy-release may be. Since, in its relations to energy, energy-release (i.e., respiration) is the opposite of photosynthesis, we should expect the opposite process of gas-exchange.

53. Is the gas exchange in energy-release (i.e., Respiration) the opposite of that in energy-storage (i.e., Photosynthesis)?

Answer by Experiments 30, 31.

EXPERIMENT 30. This may be settled by using germinating seeds and determining (*a*) whether they will grow without oxygen (which may be removed by chemical means from the chamber in which the seeds are placed), and (*b*) whether any gas they give off in growing is carbon dioxide (which may be tested by noting whether the gas given off is absorbed by such an absorbent of carbon dioxide as caustic potash so arranged that it must rise in a tube if it absorbs any gas). Prepare three large U tubes and holders as shown in Fig. 21 on the right. In the end of each place ten oat grains soaked over night (to which may be added a small wad of moist cotton or sphagnum to keep them wet, though this is not indispensable), and cork this end air-tight with a fresh rubber stopper. Half-fill each holder, the first with mercury, the second with a solution of caustic potash, the third with concentrated solution of caustic potash and pyrogallic acid.[†] Place the open ends of the three tubes in the holders, set

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[*] Respiration is introduced in this place in order that it may be studied alongside of photosynthesis; for the two processes are popularly confused and misunderstood as to their relations to one another. It would be a more logical arrangement to postpone the study of respiration until after subsections *c*, *d*, and *e* have been considered, thus allowing all cases of constructive metabolism to come together, and be followed by destructive metabolism.

[†] That the seeds are not killed by any influence exerted by the mixture is proven by two facts: first, if the mixture be removed after it has exhausted the oxygen from the tube and be replaced by water, the seeds will germinate, though more slowly than usual; second, if a bent glass tube be inserted so as to keep the chamber above the

them in darkness at a fair growing temperature, and observe and interpret results.

(The mercury tube in the above experiment is needed partly as a control, and partly to settle a certain point which comes up in the interpretation of the experiment. Water cannot well be used (though I formerly recommended it) because it absorbs carbon dioxide and rises in the tube. The U tubes must not be allowed to become wet inside or the potash will rise and destroy the seeds. Practically in the third tube it is better to place the solution of potash in the holder, and the

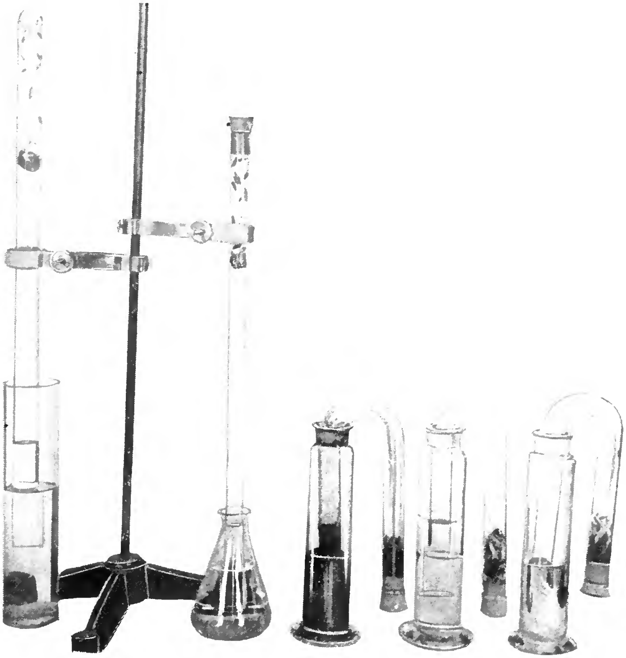

FIG. 21.—SIMPLE ARRANGEMENTS FOR DETERMINING THE GAS-EXCHANGE IN RESPIRATION.

One-fourth the true size.

mixture in communication with the air outside, the seeds will germinate, though slowly. Also, peas will germinate somewhat in the closed pyrogallic chamber as they do in the intramolecular respiration experiment (page 99), which they would not do if killed by the influence of the mixture. Removing the oxygen by phosphorus, however, seems to kill the seeds.

In the other tubes, the seeds over the potash always grow somewhat more vigorously than those over the mercury, because, no doubt, of the prompt removal of the carbon dioxide in the former case.

pyrogallic acid in the tube, which is easily effected by forcing the end of the tube down into the box of the acid. Care must be taken that the rubber stoppers are air-tight: old stoppers are very likely to leak air even when seemingly tight.)

U tubes are recommended for this experiment because they are easy to obtain, and their shape adapts them well for the purpose. Straight tubes with wire netting plugs to hold the seeds from falling can be used as shown in Fig. 21, on the left, but unless the oats are put in with great care, and all surplus water is removed from them, the water will run down and the potash diffuse up the streams and kill the seeds. In this respect the U tubes have a great advantage. Best of all, however, though rather expensive to supply in quantity, are the 150-cc. "absorption-tubes"

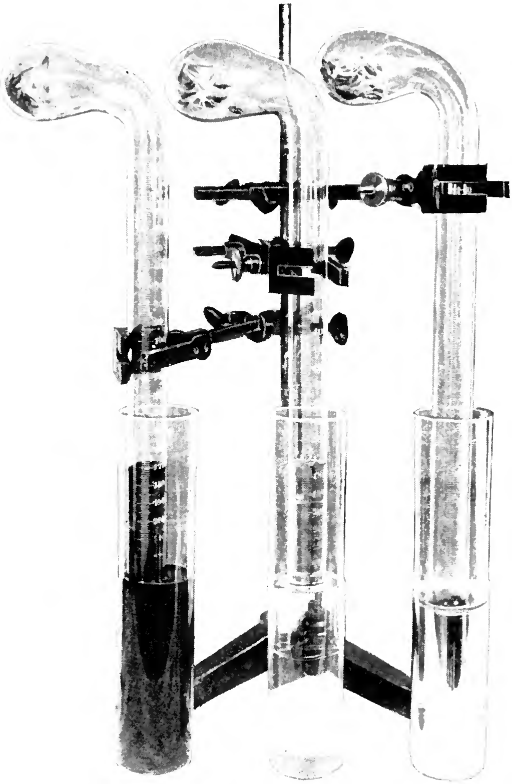

FIG. 22.—ARRANGEMENT FOR DETERMINING THE GAS-EXCHANGE IN RESPIRATION. One-third the true size.

shown in Fig. 22. When these, graduated and of known capacity,* are used, and the seeds (about thirty in number) are put in through a smaller inner tube so they cannot wet the sides of the tubes, they give the best of results. Indeed this particular experiment, using the graduated bulb tubes and the three liquids recommended (Fig. 22), seems to me to give an ideal method for demonstrating the gas-exchange in respiration, for it can be measured quantitatively and the demonstration is logically complete. Of course there should be some proportion between the size of the tubes and the number of seeds used, and I have found by experiments with oats that 1 cc. of seeds to some 20 cc. of space is very good. Of course seeds, such as peas, with pronounced intramolecular respiration (see page 99) cannot well be used for this purpose, but oats have very little of this power. (See Note 9 of the Addenda.)

The elimination of CO_2 in respiration may be demonstrated strikingly by the method given in Darwin and Acton, 2; but, as figured by them, the experiment may be simplified by having the bent tube pass through the rubber cork. An exact method using a Respirometer is described by Arthur (Laboratory Exercises, 14). One of the principal physical phenomena of respiration, the release of heat, is considered later under Growth (page 110).

What does the above experiment show as to the quantitative relations of the two gases in the exchange?

EXPERIMENT 31. Prepare two bottles with shoots as for Experiment 29. Ascertain how long a candle will burn in such a vessel; start both with pure air on a bright morning, and keep one in light as much as possible for three days and the other in darkness for that time, and test the air with the candle at the close of the third day. Use a large quantity of leaves.

It seems to be shown by Experiment 30 that seeds will not grow at all without free oxygen. This is, however, not universally true. There are other kinds (peas are an example) which are said to respire a certain amount without free oxygen.

59. Can peas respire without free oxygen?

Answer by Experiment 32.

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* Those shown in the cut are graduated for the length of a part of the tube only; if specially made, it would be better to have them graduated for the capacity of the entire bulb and tube. Of course ungraduated tubes, which are much cheaper, can be used, marks being made for the different levels of the liquids, and the capacities being subsequently determined by gradually filling the tube with water from a measuring-glass.

EXPERIMENT 32. This may be tested by placing peas in places where no free oxygen is available, and where any gas given off may be tested. Fill a small test-tube with mercury, and invert it over a small dish of mercury (as in Fig. 23); support it vertically. Soak two peas overnight, remove their coats (to get rid of enclosed air), and slip them under the edge of the test-tube so they may rise through the mercury to its top, making sure that no air enters between the cotyledons or elsewhere. Test the gas formed after a few days by slipping a piece of potash under the edge of the test-tube.

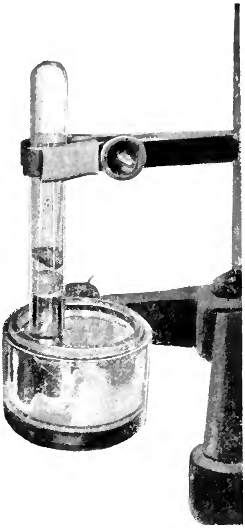

FIG. 23.—SIMPLE ARRANGEMENT FOR STUDY OF INTRAMOLECULAR RESPIRATION. (As here shown, a part of the gas is probably a decay product.) One-half the true size.

D. *Metabolism, (c) Nitrogen Assimilation.*

60. What is the part played by Nitrogen in the Plant, and whence is it obtained?

No experiment upon this subject is practicable; it is to be worked out theoretically from your various sources of information. Following are important points:

(*a*) Whence comes the nitrogen used by plants?
(*b*) In what form, where, and by what physical process is it absorbed?
(*c*) What is its chief use in the plant?
(*d*) Through what combinations does it pass in the plant, and where?
(*e*) Is free nitrogen ever taken from the atmosphere?
(*f*) How are soils nitrified?

D. *Metabolism, (d) Use of Minerals.*

61. What is the use or other meaning of minerals in the plant?

No experiment on this subject is practicable at present; it is usually investigated principally by water-culture. Answer from your various sources of information. It is important to note what minerals are absorbed and what use is made of them.

Water-culture, especially for its demonstration of the particular rôle of each mineral in the plant, is a very important subject; but I do not find it profitable for use in such a course as this. Probably it could be managed best by assigning it to some student as a special topic. The students gain some knowledge of its possibilities in their Experiment 13 earlier. Full directions are given by Darwin and Acton, and by Detmer. One of the Errera & Laurent diagrams (No. 1) gives a very effective representation of results of such cultures.

It would be profitable here to introduce experimentation upon the amount of mineral matters contained in an ordinary plant. The usual method is to weigh a shoot, dry it out completely in a water-bath, weigh again, burn it to ashes, and weigh the ash. For the precautions and details of manipulation, see Detmer, 80.

D. *Metabolism, (c) Formation of Special Substances, together with Storage, Secretion, and Excretion.*

62. What are the principal groups of special substances made by plants?

Experiment upon this subject is not profitable here; answer from your various sources of information. The substances should be tabulated to show their composition, origin, use, and mode of occurrence.

This subject, i.e., Plant metabolism, is treated as to its practical experimental study with great fulness by Darwin and Acton. Some phases of it are very easy of experiment, especially to those with some experience in chemistry.

63. What processes are involved in storage, secretion, and excretion?

What ecological phases and results are connected with these processes?

No experiment upon this subject is here practicable. Answer from your own observation and your other sources of information.

The terms secretion and excretion are not usually differentiated in their use. It seems best to apply secretion to cases in which the product has some use, and excretion to those in which it has no further use, though of course there are all gradations between these, and in most cases it is doubtful to which of the two categories a given product belongs.

Special root secretions of some importance are the acids supposed to aid the roots to take into solution otherwise insoluble substances. The nature and extent of use of these is much in doubt, but in any case the experiment usually relied upon to prove their presence, namely, the etching of a polished marble plate, is entirely inconclusive, since the phenomena of corrosion shown when roots grow upon it may be explained by the action of the CO_2 excreted by the root in its respiration.

64. Prepare a synoptical essay, of not over 400 words, upon lant Metabolism.

CORRELATED TOPICS.

Cosmic importance of photosynthesis.
Composition and conditions of formation of chlorophyll.
Photosynthesis and artificial lights.
Erythrophyll and its significance.
Chemistry of the stages in starch formation. Cases where starch is not formed as the product of photosynthesis.
Ferments,—nature and use; fermentation, and its connection with the "organized" ferments; culture-methods.
Place and stages of proteid formation.
Absorption of carbon other than as atmospheric CO_2; Mycorhiza.
Fundamental significance of the carnivorous habit.
Optimum amount of CO_2 in photosynthesis.
Corrosion phenomena.
Etiolation.

LITERATURE.

Loew, O. The Physiological Rôle of the Mineral Salts in the Plant. Bulletin 18 of the Division of Pathology, U. S. Department of Agriculture.

Ward, H. M. The Nitrification of Soils. Science Progress, **3**, 251. Vice-Presidential address in Nature, **56**, 455.

Fischer, A., translated by Jones. Chapters X and XI in Structure and Functions of Bacteria. (1900, Oxford, Clarendon Press.)

Brown, H. T. Vice-Presidential address in Nature, **60**, 474.

Kohl, F. G. Die assimilatorische Energie der blauen und violetten Strahlen des Spektrums. Berichte der deutschen botanischen Gesellschaft, **15**, 361, particularly the plate XVI.

On synthesis of albuminous substances, see note in Nature, **58**, 368.

Progress of Agricultural Chemistry. Nature, **61**, 116.

Section 2. Growth.

A. *Increase in Size.*

65. What is the approximate rate of growth in length of a vigorous shoot under normal conditions, and what fluctuations does it show?

Answer by Experiment 33.

EXPERIMENT 33. This may be tested by placing the tip of a rapidly-growing shoot, such as a flower-stalk, in connection with a self-recording mechanism whose records may be compared with records of temperature and other external conditions. Prepare an autographic auxanometer like that of Fig. 24, consisting essentially of a recording cylinder and a magnifying-wheel. From a Waterbury dollar alarmless clock of four inches diameter remove glass, face, hands, and all surplus works, leaving the central steel spindle projecting some 20 mm. above the brass frame of the works. Have turned on a lathe a hard-wood (maple is excellent) cylinder 30 cm. (a foot) long and 25 mm. (an inch) in diameter, with holes at each end a trifle smaller than the clock-spindle and exactly centered by the lathe, so that the cylinder may be forced gently down upon the spindle and revolve exactly vertically above the clock, on which it will turn once an hour. Cover the cylinder with the smoothest obtainable paper (if highly glazed paper is used, it will go on without wrinkles, but an unglazed paper will fit better if put on wet), fastening it by mucilage along the free edge, which must turn in a direction not to catch the pen as the cylinder revolves. Have turned on a lathe (from maple) a magnifying-wheel, forming four concentric wheels of different sizes side by side in one piece (Fig. 25), the outer 12 cm., the next 6 cm., the next 3 cm., and the smallest 1.5 cm. in diameter. All are to be as thin as they can be turned, and grooved on their rims. A very small hole, a little larger than a coarse needle, is to be turned exactly in their common axis, and the whole is to be covered with a thin coat of shellac to prevent warping. A support for the wheel is made by fixing (with sealing-wax if necessary) a perfectly bright slightly-vaselined needle in a piece of capillary tubing, and the wheel should turn freely upon it; the tubing is then to be supported horizontally on a tripod support with the wheel vertical. A recording-pen is to be prepared from small glass tubing drawn to a capillary point and bent at right angles as shown in Fig. 24 (though it should be of much smaller size than there shown); it is held in a loop of flexible brass to which a thread can be attached; into the pen a few drops of chronograph ink are drawn. Set the wheel with one edge over the cylinder, and the other

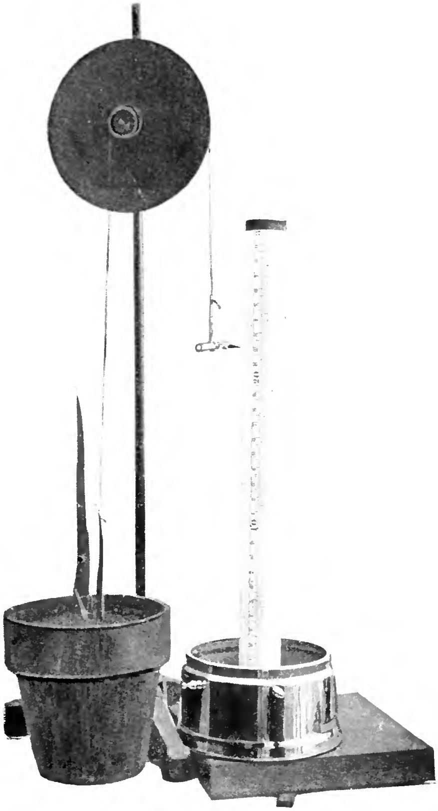

FIG. 24.—AN AUTOGRAPHIC AUXANOMETER. (As here shown, the pen is much too large.) One-third the true size.

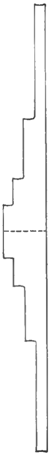

FIG. 25.—MAGNIFYING-WHEEL, IN SECTION. One-half the true size.

over the tip of the shoot. Fasten, by a small drop of glue, to one of the smaller wheels the end of a silk thread thoroughly waxed (to lessen changes in length through hygroscopic changes) and make several turns around it. To the groove in the larger wheel fasten also a similar thread which makes a turn in the opposite direction. Attach the free end of the first thread to the tip of the part of the plant to be measured, and the end of the other to the recording-pen, which can be made to press against the cylinder by giving it several turns in the wrong direction. Thus arranged, the wheel will turn as the plant grows, and the pen will trace a spiral line on the revolving cylinder. The exact amount of growth through the 24 hours can then be read off for every hour by placing a millimeter scale along the cylinder from the starting-point downward (or on the paper removed from it), on which of course the record appears magnified in proportion to the comparative diameters of the wheels used. It is well to try one record through the 24 hours in a thermostat kept at constant temperature, and another in the ordinary fluctuations of the greenhouse, temperature being read from the thermograph. Particularly good for the study of growth are vertically and rapidly elongating flower-stalks, such as those of the hyacinth or grape-hyacinth. The paper is to be removed from the cylinder for preservation, and a curve is to be plotted from it.

The advantage of the quadruple over a double wheel (such as is used in Fig. 24) is that it allows of different degrees of magnification, and hence makes the apparatus available for other purposes where continuous records are needed. Owing to the leverage exerted by the pen when placed on the largest wheel, it tends to exert a considerable pull upon the plant, thus affecting its growth; hence the pen should be made as small and light as possible. For exact work, it would be better to use a counterweight to balance the pen (using a close coil of copper wire which may easily be made the same weight as the pen), and to make the wheel turn by a small weight placed on the thread coming from the plant and passing over (and once around) the smaller wheel.

(The cylinder should be of hard wood, since weight is not a drawback in this case, and the holes for setting it on the clock-spindle will not wear loose as with soft wood. When set revolving on its axle, the wheel should come to rest indifferently at any point. If it does not, small pieces of paper can be gummed to the lighter side until it balances. The clock must be wound and a new record commenced every twenty-four hours, but a vigorous plant will cover the cylinder with its magnified record in that time. For some purposes an eight-day clock would be better. Watering the plant introduces an error, as it swells the soil and lifts the plant a trifle; hence the watering should be done just before the new record is begun. Some practice will be nec-

essary to secure a good pen, but a few trials will give success. The capillary tip of the pen should be made smooth by rubbing it on a fine whetstone or piece of ground glass, on which it should be rubbed at such an angle that the point will rest at right angles to the cylinder. Or, with great caution, it can be smoothed in the gas-flame. These pens can be made to write with any desired degree of fineness, even down to a scarcely visible line,—the finer the point is drawn out the finer the line, but such fine pens need a smooth paper. It is better to make a number of pens and choose the best. It may be filled by connecting the large end with a pipette by means of small rubber tubing ; by forcing a little air from the pipette with the tip of the pen in the ink as the bulb is allowed to expand, the ink will enter the pen ; a few drops are sufficient (or the bit of rubber tubing alone may be used on the same principle). If the brass loop (made from a brass paper-fastener) grasping the pen is made a little small, the pen may be forced into it and be tightly gripped. The thread may be attached to the shoot by tying under the uppermost bud or pair of leaves ; if a vertically growing leaf is used, the thread may be put through a tiny hole in its tip. The weight of the pen should be so adjusted that the least possible pull will be exerted upon the plant. To set the cylinder truly vertical, or in other words the clock truly horizontal (great care must be taken in removing the surplus parts from the clock not to bend the works), use a small thick oiled board (Fig. 24) set level, as tested with a spirit-level, by tapping small wedges under it. The cylinder must be forced down firmly on the spindle or it will wobble. Where there is not room for plant and clock under the same wheel, the former may be set at a little higher level, or else the plant may be set at a distance and the thread led from it to the wheel through a ring of glass tubing or over a needle so arranged that the thread will be vertical from plant to loop or needle and will then reach the wheel in the plane of the latter; but all threads should be as short as possible in order to avoid hygroscopic changes in length. Half-hour records may be obtained from the cylinder by cutting out one-half of the paper vertically and joining the edges of either piece. It is rather convenient to have a millimeter scale stamped vertically on the paper.

The above-described apparatus forms an autographic machine which should be utilizable for other purposes as well.

Numerous forms of auxanometers have been invented, from the very accurate one of Sachs, familiar in its many modifications, through numerous published cuts, down to forms so crude as to be of slight worth. Pfeffer's modification of Baranetzsky's instrument, made by Albrecht of Tübingen, is the best obtainable form. A very exact instrument is described by Frost in Minnesota Botanical Studies, No. XVII, 1894 ; a crude form is given by Bumpus in Botanical Gazette, **12**, 149, and a better one by Barnes in the same volume, 150; another is described by Stone, Botanical Gazette, **17**, 105, and another in the same journal, **22**, 258; another is given by Golden, Botanical Gazette, **19**, 113, (for growth in thickness,) and another by Arthur, Botanical Gazette, **22**, 463. Corbett has described another form in the Ninth and Twelfth Annual Reports of the West Virginia Agricultural Experiment Station.

66. By what mode of expansion (in what parts) do leaf, root, and stem increase in size?

Answer by Experiments 34, 35, 36.

EXPERIMENT 34. For leaves this may be tested by marking very young ones into regular areas and observing the changes in form and size of those areas during growth. With a rubber stamp marking small squares, stamp the upper surfaces of very young leaves, holding them against a cork to secure a firm flat surface, and observe subsequent changes in the markings.

Of course the stamp must be made to order, but it is inexpensive; one marking 45 (9 × 5) squares of 3 mm. on a side is excellent; the lines must be fine; a black stamp-ink should be used, and water not allowed to touch the leaves.

The same result may be obtained by pushing pins at regular intervals through a piece of sheet cork, with points projecting a little, and pressing these points through the leaf, which is held against another piece of cork. The spread of the holes will give a record of expansion of the leaf, but not so effectively as in the case of the stamp. Regularity in position of the pins may be secured by putting them through the angles of a piece of cross-section or plotting-paper placed on the cork.

EXPERIMENT 35. For stems this may be answered by marking very young ones at short and regular (about 2 mm.) intervals from the tip backwards by water-proof India ink applied with a stretched thread (see page 39), and observing their rate of separation. Stems as free from leaves as possible should be selected.

EXPERIMENT 36. For roots this may be answered after the principle used for stems. Start a few horse-beans in wet sphagnum, and when the roots have developed to the length of an inch, mark one or more of them, at intervals of 2 mm. from the tip backwards, with water-proof India ink applied by a stretched thread. Fasten the seed to the cork of a flower-pot moist chamber (see page 118) with the root pointing directly downwards, and after a day or two note the positions of the marks.

The roots may also be grown in thistle-tubes, as sometimes recommended, on the outside of which the absolute growth may also be recorded, but in my experience the method is inferior to that described above.

67. Where in the most highly differentiated plants is growth localized or most active?

Your studies in earlier courses have given you data for answering this question; review the facts, supplement from other sources, and express conclusions in a diagram showing the growth areas of a higher plant shaded in proportion to the activity of each.

B. *Histological Differentiation and Assumption of Form.*

68. What are the factors controlling histological differentiation and assumption of form?

No experiment upon this subject is practicable; answer from your various sources of information. Particularly note the processes concerned in the maturation of embryos and vegetative points.

C. *Effects of External Conditions.*

69. In what way is growth directly (i.e., non-irritably) affected by the principal external physical agencies?

a. By presence, absence, and different qualities of light?

Answer by Experiment 37.

b. By differences in temperature?

Answer by Experiment 38.

c. By electricity?

Answer from study of the references below.

d. By moisture in various degrees?

Answer by Experiment 39.

EXPERIMENT 37. This may be tested by using parts in which growth occurs uncomplicated by photosynthesis, as in germinating seeds, and compelling them to grow under light of different colors. Prepare an even-moisture germination apparatus as shown by Fig. 26 (consult page 44). In each of the four Zurich germinators sow ten soaked seeds of oats and cover respectively with (*a*) clear glass, (*b*) glass blackened by asphalt varnish, (*c*) red glass, and (*d*) blue glass. Set the apparatus in bright though not direct sunlight, and compare the rates (*a*) of germination, (*b*) of early growth, and (*c*) of later growth. Express results quantitatively in your records.

Colored glass is most convenient for this experiment, but none is obtainable in which the colors are spectroscopically pure. Gelatine plates are better (see earlier, page 89), and of course must be used attached to glass. But very much better results may be obtained

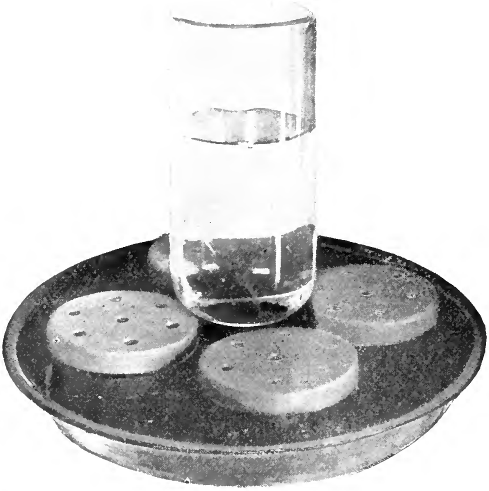

FIG. 26.—GERMINATION APPARATUS AUTOMATICALLY WATERED.
One-fourth the true size.

by use of pure-color chambers constructed thus (Fig. 27): Take five Soykas flasks, which have their faces ground and polished flat, and fill them with five colors thus: white, obtained with distilled water black, obtained with distilled water and India ink; red, obtained by the dye "scarlet"; blue, with ammoniacal copper sulphate; green by a mixture of potassium chromate and ammoniacal copper sulphate (the latter with an excess of ammonia). In the three latter cases the mixtures must be tested in each flask with the spectroscope, and made of just such a density that they cut out all colors but their own, but the least possible of the latter. Thus adjusted, loss by absorption and reflection being about equal in the three, the proportional intensity of the three colors transmitted by the flasks must be about as in sunlight. The rounded edges of the flasks should be blackened (with black paint) to prevent light passing through them. Select now an eight-inch porous earthenware pan or flower-pot (see Fig. 27); cut a round glass plate just covering it. Cut from the felt paper used in herbaria two circles the size of the glass, and from these cut five circles equidistant from one another, a little smaller than the Soyka flasks. Glue these papers with the circles matching to the two surfaces of the glass, and blacken the edge of the glass. Select five two-inch flower-pots, and pack them with sphagnum in the pan at such distances apart that they will match the open circles on the glass cover and will project a trifle above the rim of the pan or pot. If now the cover be adjusted with the color flasks in place, there will be beneath them five chambers, receiving no light except of the pure color, readily kept moist (by water placed in the saucer under the pan and soaking up to the pots through the moss) and ventilated through the holes in the bottoms of the pots. If in these small pots seeds be placed, and a strong light allowed to fall upon them, the comparative growth under the colors may be determined. Particularly advantageous for this are mustard-seeds. If these be soaked, and ten be placed near the top of each pot (to which they will adhere by their own mucilage), they will grow vertically and their growth may be exactly measured. The light should be thrown as nearly vertically upon them as possible, either by special arrangements, or by fastening the flasks in place with gummed paper, and tipping the apparatus to the proper angle. (A thorough watering at the beginning of the experiment is all that is needed.)

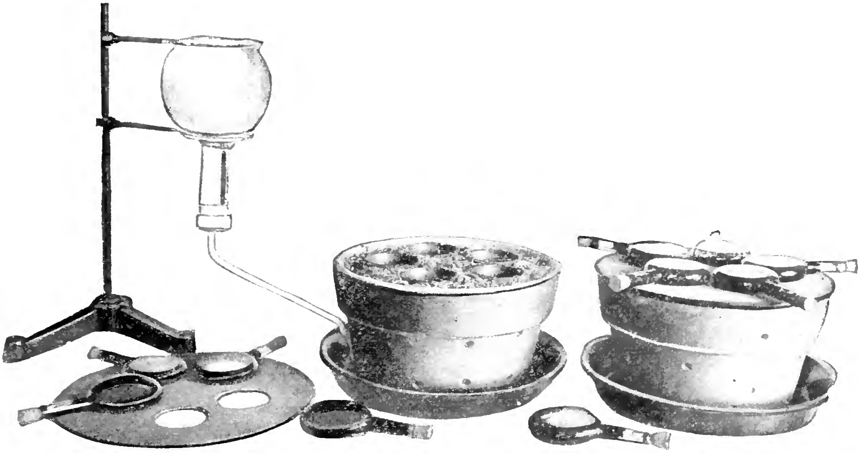

FIG. 27.—PURE-COLOR CHAMBERS, THE MIDDLE ONE WATERED BY AN AUTOMATIC ARRANGEMENT.
One-fourth the true size.

This arrangement gives pure-color chambers available for other purposes, such as photosynthesis and heliotropism. For special purposes it could be made much larger, and the light thrown directly down upon it from above with a mirror. If the tops of the smaller pots are ground smooth upon a revolving emery-wheel attached to a water-motor (or a grindstone), they will fit perfectly against the paper of the cover. The advantage of this felt paper is that it allows a practically light-tight joint with the flasks. It is well to bore holes in the sides of the larger pot or pan to allow better ventilation.

A source of error in the apparatus which must be guarded against is the difference of temperature under the different colors. My tests to determine this show that the difference increases with the intensity of the light. If the light is not direct intense sunlight, the differences are insignificant, and with very bright light the differences may be rendered insignificant by covering the whole with thin tissue-paper. For special work even these differences could doubtless be removed by keeping up a circulation of air through the pots by rubber tubes from each connected with an aspirator.

EXPERIMENT 38. To test this it is only necessary to place soaked oats in covered Zurich germinators or flower-pot saucers, each of which rests in a saucer containing water, and to stand them in darkness in places known to be of different temperatures. (See Note 10 of the Addenda.)

EXPERIMENT 39. Invent a simple method of testing this.

D. *Physical and Chemical Processes.*

70. What chemical processes are involved in Growth?

No experiment is here practicable upon this subject, aside from that already taken up in connection with Respiration (Section 58, Experiment 30). Answer theoretically from your various sources of information.

We next consider what physical processes are involved in Growth. Of these, its relations to the absorption or release of heat, and its relations to amount of mechanical work as manifested in exertion of pressure, etc., occur for investigation.

71. Is heat either absorbed or eliminated in processes connected with Growth?

Answer by Experiment 40.

EXPERIMENT 40. This may be settled by comparing the temperature of growing (and, therefore, respiring) with that of non-growing (non-respiring) tissues placed under the same conditions. Fill a three-inch flower-pot with peas soaked overnight, and another like it (for a control) with soaked peas freshly killed by hot water. Invert them both over dishes containing a solution of caustic potash (to absorb all CO_2 formed, and thus allow a more active respiration), keeping the peas from falling out by wire netting or the tops of Zurich germinators (Fig. 28). Thrust accurate thermometers through the holes of the pots into the peas; cover both with close non-conducting hoods made of felt paper (Fig. 28), set in a favorable place for growth, and observe the thermometers minutely every few hours.

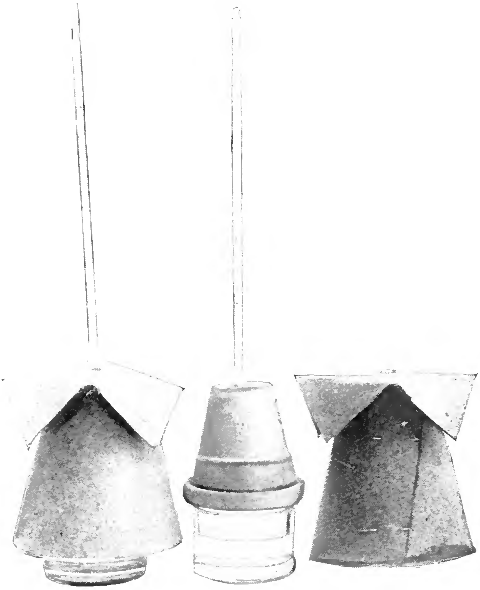

FIG. 28.—SIMPLE ARRANGEMENT FOR STUDY OF HEAT-RELEASE IN GROWTH. One-fourth the true size.

What is the precise physical and physiological meaning of the phenomenon shown?

This may also be well tested by placing many rapidly-developing flowers in a flask or wide-mouthed bottle, and noting the difference between the temperature of a thermometer thrust among them in comparison with one in a similar vessel lacking the flowers. Of course this must not be carried on in direct sunlight. Another excellent method is that of placing a thermometer in the spathe of a rapidly opening araceous flower such as Richardia or Symplocarpus, and comparing it with another immediately outside. Since the temperature-differences are slight, the more minutely the thermometers read, the better. An elaborate and more exact method of investigating this subject is given in Annales des Sciences Naturelles, Botanique, 1893.

72. Can mechanical resistance, or pressure, be overcome in Growth, and if so, to what extent?

Answer by Experiment 41.

> EXPERIMENT 41. This may be settled by placing young growing parts under conditions requiring them to overcome measurable pressure in order to expand. Start six horse-beans in sphagnum in the germination-boxes. When the roots have reached 3 or 4 cm. in length, fit to each one near the cotyledons a cork pressure-jacket made as follows: Take small corks of about 1 cm. diameter and one half that length, and through their axes burn holes, about the diameter of the hypocotyls of the beans; split each cork lengthwise into two equal halves, and make all smooth. Around each cork place a rubber band whose tension is such, as determined by hanging weights to one of the halves, that the six corks form a series requiring tensions of from about ten to about five hundred grams to force them apart. Place these corks upon the beans, replace the latter in the sphagnum, and examine from time to time to determine which of the jackets are forced apart, and hence what pressures can be overcome.

The epicotyls of young Ricinus plants give good results with these jackets.

Do you know of any cases in Nature where mechanical pressure is exerted by growing parts?

What is the probable physical basis of this power to overcome resistance?

E. *Movements.*

A familiar fact about Growth is that it is accompanied by movements other than those of mere increase of size. It is important to ascertain whether these are determined by internal or external influences, or by both. Some of these movements are plainly responses to light, gravitation, etc., and these are to be studied presently under the section on Irritability. The question then remains whether there are movements connected with the process of Growth itself and independent of external stimuli. One way to test this is by determining whether, in elongating structures, when the one-sided influence of light, gravitation, etc., is shut out, the growth is in straight lines.

73. Is the growth of elongating structures, apart from responses to lateral external stimuli, exactly in the line of their axes, or does it show lateral movements?

Answer by Experiment 42.

EXPERIMENT 42. This may be determined by placing a plant in such a position that the chief external stimuli, light, gravitation, heat, and moisture, cannot exert a lateral stimulus upon its elongating stem, and by arranging a system of sighting along the axis of the stem so that the smallest deviation from a straight line will be made visible. The former may be accomplished well enough for all practical purposes by placing the plant (one growing rapidly with a vertical stem) on the table of a well-lighted greenhouse, and surrounding it by a black-paper cylinder which rises three or four inches above its top. To accomplish the latter, some method must be employed which will both allow the exact direction of the axis to be sighted, and also will magnify the slightest lateral movement, and permit it

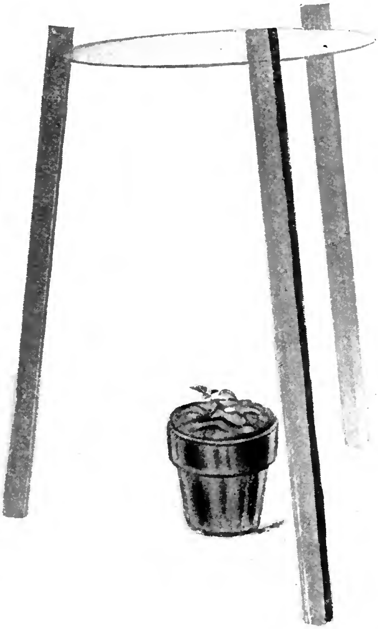

FIG. 29.—SIMPLE ARRANGEMENT FOR THE STUDY OF CIRCUMNUTATION. One-fourth the true size.

to be recorded. Prepare a recording glass supported by a tripod as shown in Fig. 29, the glass being some ten inches above the plant. Prepare the slenderest glass filament (see page 39) you can, 2 cm. long; place a tiny bead of black wax on one end, and slip about a half cm. up the other a tiny circle of white paper with a tiny black spot in the middle through the center of which passes the filament. Attach this filament vertically to the side of the top of the stem with shellac, bead upward. Sight through the glass plate, bringing the bead and black spot into line, and, exactly on the line of sight on the plate, make a mark with a very fine glass pen made by drawing a glass tube to a capillary point. Chronograph ink should be used. Record three or more times a day for two or three days, joining the marks and marking the times. On some single warm day, record at intervals of ten minutes for two or three hours. The records may be traced from the glass by use of tracing-linen.

Another, somewhat different, and more exact method of securing the record is given by Stone in Botanical Gazette, **22**, 262.

Usually the points obtained in the observations are joined by straight lines, but the resulting angularity conveys a very wrong impression of the real nature of the movement; hence I think it better to join the marks by sweeping curves of as limited radius as possible; the result will be at least as correct as the straight lines and much more true to nature.

74. Do these autonomic (in this case Circumnutation) movements occur in connection with other growth movements?

Answer by Experiment 43.

EXPERIMENT 43. The bending movement made by seedlings in lifting their cotyledons from the ground may be utilized in testing this. Plant in a pot of earth three or four Lima beans (using several in order that some one may be pretty sure to be in proper condition when wanted), and when the bow of the hypocotyl of one of them appears just above the ground, attach to its highest point the filament and circle used in the preceding experiment, and record on the glass the movements during the straightening of the hypocotyl. As this takes place rapidly, it is needful to record often.

75. How widespread is the circumnutation movement, and what is its fundamental significance?

Answer from your various sources of information.

76. What are the other known autonomic movements?

Answer from your various sources of information.

77. Prepare a synoptical essay, of not over 300 words, upon Growth.

CORRELATED TOPICS.

Ontogenetic unfolding of the higher plant, and factors determining assumption of form; hereditary, direct, and irritable factors.

Internal factors; polarity, rectipetality, epinasty, hyponasty, Sachs' curvature, correlations.

"Vitality" and its nature, particularly in seeds, and "resting periods."

Grand periods, annual periods and rhythms.

The cardinal points in growth, minima, optima, and maxima: rigors.

Is temperature a "stimulus" or a "condition" in growth?

Tissue tensions and their relations to growth, and assumption of form.

Spontaneous movements of Desmodium, etc.

Contractility of roots, torsions, fasciations.

Growth in darkness: etiolation.

LITERATURE.

Darwin, C. Power of Movement in Plants. (Read at least the Summary.)

Anderson, A. P. The Grand Period of Growth in a Fruit of Cucurbita pepo, determined by Weight. Minnesota Botanical Studies, No. XXI, 1895.

Electro-culture. Nature, **61**, 602. Also Stone and Kinney in Hatch Experiment Station Bulletin, No. 43, 1897.

Section 3. Reproduction.

The physiological phenomena connected with Reproduction, extremely important though they are, do not admit of profitable experimental study in such a course as this. Some of them have already been studied in your earlier courses; a few are taken up under other sections, while others merge into purely ecological investigations. It is necessary, however, to acquire a knowledge of the leading facts, and clear ideas upon the leading principles, of the main divisions of the subject.

78. What are the essential facts and theories in the physiology of Reproduction?

Answer very synoptically from a review of your knowledge earlier acquired, and from your various other sources of information. Following are important topics.

(*a*) What is the fundamental relation of reproduction to growth?

(*b*) What are the probable origin and phylogeny, and the physiological characteristics of asexual reproduction?

(*c*) What are the probable origin and phylogeny, and the physiological characteristics, especially the "advantages," of reproduction by fertilization?

(*d*) What is the origin and significance of cross-fertilization, and what mechanisms has it resulted in?

(*e*) What is the nature of variation, heredity, rejuvenation; and what is the nature of the physiological connection between parent and offspring?

(*f*) What is the known physical basis of these processes (i.e., in cell-division, fertilization, etc.)?

(*g*) What is the relation of the ontogeny to the phylogeny of an organism?

(*h*) What is the physiological significance of alternation of generations, parthenogenesis, xenia, results of grafting?

Section 4. Irritability.

It is a matter of every-day observation that plants, while inheriting the general characteristics of their forms, have a large power of accommodating the details of size, shape, texture, etc., to the conditions of their immediate environment. This power is termed Irritability. It is necessary now to investigate the precise methods by which this accommodation is brought about, and to become acquainted with its common phases or manifestations. The important environmental influences are, as is well known to you, gravitation, light, heat, moisture, and there are many others of lesser importance.*

A. *Geotropism.*

It is a familiar fact that most land plants in growing bring their main roots to point downwards, their main stems to point upwards, and their side roots and stems into intermediate positions. We must now seek to find out what determines those positions.

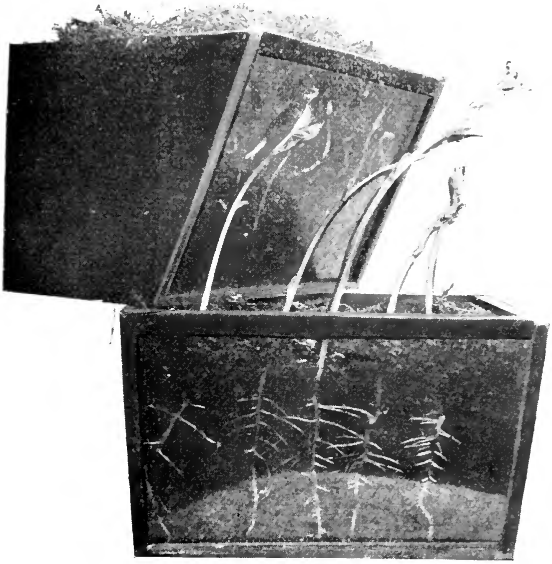

FIG. 30.—GERMINATION-BOXES. One-fourth the true size.

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* Upon Geotropism, as well as upon other phases of Irritability, an immense amount of valuable experimentation is practicable, enough to fill the entire year. It is needful, therefore, in order to keep the subject within its proper place and proportion in this course, rigidly to choose only the most important and illustrative topics. Accordingly those are here selected which seem to demonstrate best the true nature of Irritability and its leading manifestations, especially those of ecological importance. Geotropism is much more fully treated than the others, because, by means of it, the different factors concerned in irritable responses may be so well differentiated and determined.

79. Is the direction of growth of primary root and primary stem guided by internal or by external influences?

Answer from Experiments 44 and 45.

EXPERIMENT 44. This may be tested for roots, by planting a number of seeds in as different positions as possible, and observing the positions the roots take in issuing from them. Near the top of a germination-box (Fig. 30) filled with sphagnum, plant five or six soaked horse-beans in as different positions as possible. Keep the whole dark and moist and observe positions taken by the roots.

Another excellent method is the following. Prepare a moist dark-chamber thus: Select a five-inch (or larger) flower-pot and drill out the bottom (easily done with a hammer and screw-driver), in place of which a cork is to be fitted (Fig. 31). Pin to this five or six seeds in different positions and bring them out to the heads of the pins; surround them with sphagnum kept wet; place the cork in the pot and invert the latter in a saucer of water. The roots grow excellently in such a moist-chamber. After the roots have attained a length of 2 or 3 cm. invert the cork under another pot, and observe effect upon the roots. A flower-pot stood in a dish of water makes the best of moist-chambers.

FIG. 31.—MOIST-CHAMBERS FOR GEOTROPIC EXPERIMENTS.
One-fourth the true size.

An ordinary funnel to supply the sloping glass surface is a fair substitute for the germination-box.

In all such experiments it is necessary to use a seed in which the hypocotyl does not elongate and raise the cotyledons; hence horse-beans or corn are very good.

Experiment 45. This may be tested for stems by observing the positions taken by those coming from the seeds in the preceding experiment, and also by placing the stems of growing plants in new positions. Select three small similar plants each with a single actively growing stem, and place them in darkness in such positions that their stems will point in the most different possible directions, and observe results.

(*If the pots are to be turned upside down, the earth may be kept from falling out by covering it with a wad of sphagnum held in place by strings across the pot.*

80. When the direction of growth is once determined, is it held regardless of external conditions, or does it continue responsive to the original condition determining it?

Answer by Experiment 46.

Experiment 46. This may be tested by changing the positions of roots after they have attained to some size. After the roots in Experiment 44 have reached a length of two or three inches, and side roots have appeared, turn the box in the plane of the glass through 45°, and observe effect upon the position of the old and the new roots.

Also, after the stems of Experiment 45 have shown their complete result, restore them to their former positions.

The results of the preceding experiments show not only that roots and stems take definite positions in growth which are independent of the position of the seed or plant from which they arise, but also that unequal distribution of light, heat, moisture, etc., cannot be the influence which guides them into those positions. The suggestion then arises that their position is perhaps connected with the "up-and-down" direction, which of course is determined solely by gravitation. Obviously this hypothesis could be tested if a set of growing parts, with other conditions as in the preceding experiments, could be removed from the influence of gravitation.

81. What is the effect upon the position of roots, when, with other conditions as uniform as possible, gravitation is not allowed to act upon growing seeds?

Answer by Experiment 47.

Experiment 47. Since nothing on the earth's surface can be removed from the influence of gravitation, the only way in which it can be kept from acting unilaterally upon an object is by making it equalize itself by a constant and uniform revolution of the object in a vertical plane. Select two good corks some 12 cm. in diameter, and pin to each, in as different positions as possible, some five good soaked horse-beans, bringing the latter away from the cork to the heads of the pins. Surround and cover both with moist chopped sphagnum, and over each cork place a crystallizing-dish about 6 cm. deep, of a size proper to hold tightly when pressed over the bevelled edge of the cork. Attach one of the corks to the disk or arm of a clinostat, and keep it revolving in a vertical plane once in fifteen minutes (to allow a complete revolution within the reaction-time of the roots), and set the other in a fixed vertical position (see Fig. 32). Compare from day to day the positions taken by the roots on the two corks.

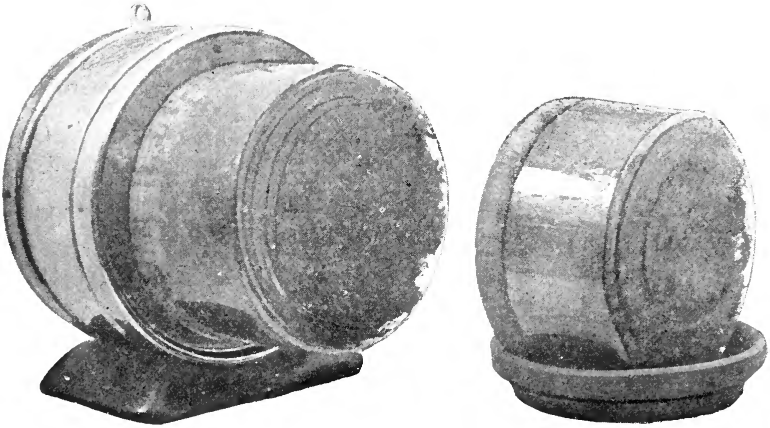

FIG. 32.—A SIMPLE CLINOSTAT.
One-third the true size.

A clinostat is necessary for this as for other physiological work, and is simply indispensable for any physiological laboratory. That most used is Wortmann's, costing about $60, which may be imported through any dealer in scientific apparatus. A more expensive one is Pfeffer's, made by Albrecht, costing about $100, while one recently described by Hansen (in Flora, **84**, 353) is advertised at 115 marks ($29). A simple form run by a water motor is described by Stevens (Botanical Gazette, **20**, 92), and another run by clockwork by Stone (Botanical Gazette **22**, 259). A comparatively inexpensive and, for purposes of investigation of the *principle* of geotropism, heliotropism, etc., entirely efficient clinostat may be made as follows (see Fig. 32): Procure a Seth Thomas eight-day clock (costing about $6), and have a watchmaker remove hands, face, and surplus works, and gear it down so that the minute-hand spindle turns once in fifteen minutes. This may be accomplished partly by shortening the hair-spring and partly by removing the alternate teeth from the escapement-wheel. A brass sleeve about an inch long, tightly fitting over the spindle, is to be made, bearing at its outer end a brass disk two inches in diameter, perforated with a few holes on its margin and revolving in a plane parallel and close to the clock-face. A glass plate with holes for the spindle and for winding should then be placed over the works, or the original face may be retained. Such a clinostat will carry easily a considerable weight in a vertical plane, and a five-inch pot when revolving horizontally. In the latter case a saucer or crystallizing-dish must always be placed beneath the pot to prevent water from running from the pot into the works. The clock will need winding once in two days, and while the cork is removed for the purpose, it should be kept slowly revolving in the hands.

The result of an earlier experiment (No. 46) showed that side roots are guided, though in a different direction, by the same influence which guides the main root. The question now arises as to how the two can respond so differently to the same influence, and to determine this we need first to find out whether the position taken by the side roots is in any way correlated with that of the main root or is independent of it.

82. Is there any correlation of position between the main and the side roots?

Answer by Experiment 48.

EXPERIMENT 48. To answer this it is only needful to remove the tip of the main root, and observe whether the side roots remain unaffected. After the growing roots in Experiment 44 show small side roots, mark their positions upon the glass with waterproof India ink. Cautiously slip the glass up in its groove until the main root-tip is exposed; cut this squarely and cleanly off 5 mm. behind its end, and replace the glass; observe effects upon the growth of the nearest side roots.

If Experiment 44 has been tried with the moist-chamber instead of the germination-box, the tips of the roots may easily be removed, though the subsequent growth of the side roots cannot be so accurately determined as with the germination-box where their course may be marked upon the glass.

83. In what part of the root does the geotropic response normally take place?

Answer by Experiment 49.

EXPERIMENT 49. This may be determined by so marking a geotropically-responding root that the place where growth is most active becomes evident; this has already been done for another purpose in Experiment 36, but it should be here repeated with the marked root placed horizontally. Along with it should be placed another similar root also fixed horizontally, but marked with rings. Observe results after a few hours.

A correlated question of much importance is this: If response is prevented in one place, can it take place in another? Experiment upon this subject is not easy; but I have had fair success by using horizontally-placed roots with small glass caps made just long enough to cover the tip and the usual growth zone (following the general method of Czapek mentioned in the next section).

84. In what part of the root does the perception of the gravitation-stimulus take place?

This may be determined by means of the extremely beautiful and conclusive experiment devised by Pfeffer and Czapek,* described by the former in the Annals of Botany, **8**, 317. The method is described also by Darwin and Acton, 174, but is rather too difficult for use here.

85. Are there cases in which the geotropic response is localized in specially differentiated structures?

Answer by Experiment 50.

EXPERIMENT 50. Select two fairly well-grown grass stems (or stems of a greenhouse Tradescantia), and cut from each a piece 10 to 12 cm. long, containing at about its middle one of the swollen nodes. Fill a small flat wide-mouthed bottle with wet loose sphagnum. Push the lower end of one of the pieces of grass horizontally into this (Fig. 33). Push the two ends of the other piece into glass tubes brought close enough to one another to allow only the node and the bottom of the stem to remain uncovered; bind these tubes with rubber bands to another piece of tubing so they will not bend with the stem, and thrust the lower end into the sphagnum, which will keep both pieces wet. Cover with a bell-jar and darken (to eliminate possibility of light-responses), and observe results particularly upon the form of the node.

Another somewhat different way of holding the node in position is

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* Czapek's original paper is in Jahrbücher für wissenschaftliche Botanik, **27**, 243; see also **35**, 313.

given by Darwin and Acton, and in principle has been used in recent researches by F. G. Kohl (Botanische Zeitung, 58, 1).

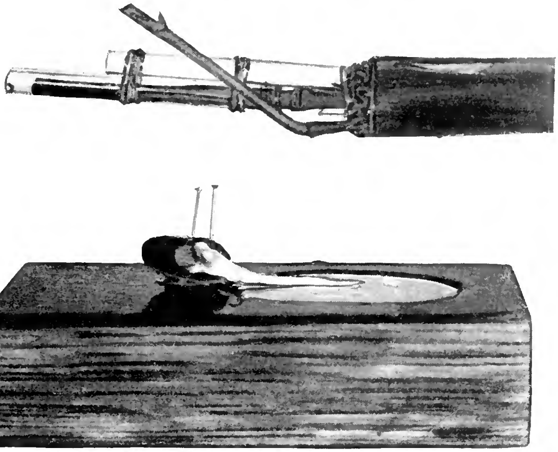

Fig. 33.—Simple Arrangements for Study of Geotropic Bending. One-third the true size.

86. What effect is produced upon the growth-processes when geotropic stimuli are allowed to act but growth-response is prevented?

Answer partly from Experiment 50, and from Experiment 51.

Experiment 51. This may be tested by placing a positively or negatively geotropic growing part, such as a shoot, horizontal, preventing it from bending geotropically and later suddenly releasing it. Invent a way of accomplishing this.

87. Is the geotropic response simply mechanical and passive, or can it take place against resistance?

Answer by Experiment 52.

Experiment 52. This may be tested by placing a geotropically-growing part, such as a root, in such a position that, in order to continue its growth downwards, it must exert a distinct, and preferably measurable, pressure. Such a resistance-measurer in which the flotation-power of mercury is utilized is shown in Fig. 34 on the left. Select a large upright perfectly clean test-tube, and a vial (which is lighter than glass tubing of the same diameter) somewhat smaller in diameter than the tube. Remove (by the method described for large tubes on page 38) the lower end of the vial, and fit into both ends tight narrow corks. Through the diameters of each cork force two fine needles at right angles to each other cut to such a length from the points that they are slightly shorter than the diameter of the test-tube. (Or the bottom may be left in the vial, and the needles attached to it by sealing-wax.) Such a vial will float upon the top of mercury in the test-tube, with the needle-points against the glass holding it in an upright position, and allowing it to slip up and down in the mercury practically without friction. In the top of the upper cork, left considerably longer than

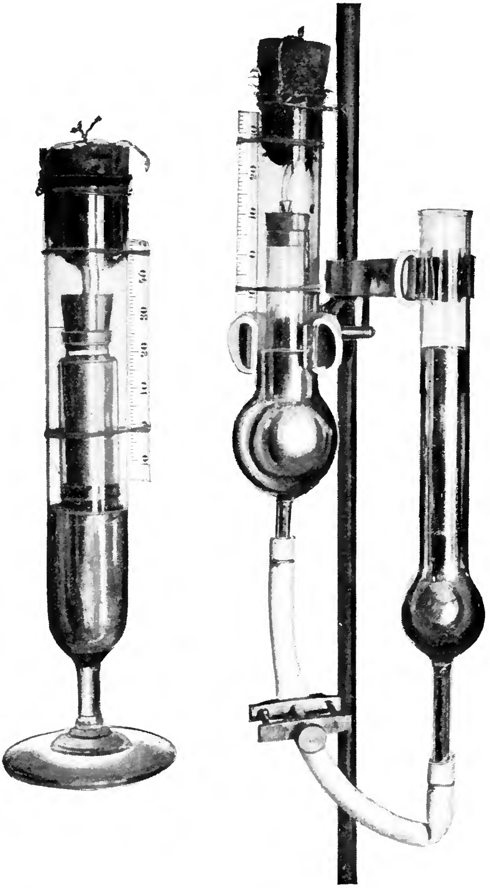

FIG. 34.—ARRANGEMENTS FOR DETERMINATION OF GEOTROPIC PRESSURE OF ROOTS. One-half the true size.

the lower, should be smoothly burnt a small hole to receive the tip of the root (so that the latter may not nutate from the center of the cork). Place some soaked horse-beans in moist sphagnum with hypocotyl pointing directly downwards, and leave until the roots are nearly 2 cm. long. Pin one of the stoutest firmly to the under side of a cork fitting the test-tube; in the cork vertical grooves are to be filed to admit air. The root should point vertically downwards, and the seeds should be wrapped in moist sphagnum. Place just so much mercury in the test-tube that, when the cork is in place and fastened with wire, the tip of the root rests lightly inside the hole burned in the upper cork. Set the apparatus in a dark and moderately-warm place, and observe frequently. An attached scale, opposite which one of the needle-points moves, allows the exact amount of depression of the float into the mercury to be observed. When the root has pushed down the float to a point at which a lateral bending of the root begins, the point should be noted. The root is then to be removed, and gram weights are to be placed upon the float until the latter is depressed to the same point to which the root depressed it. The power of the root to grow vertically downwards will thus be given exactly in grams. The readings should be taken at the same temperature at which the experiment is started.

The above arrangement gives a very exact and practical method of measuring these pressures, and it can doubtless be adapted to other purposes (see page 45). With it I have obtained pressures of over 30 grams with bean roots, before they bend. To overcome the slight difficulty of securing the proper height of mercury in the tube, the arrangement in Fig. 34 on the right is good. By sliding the right-hand tube up and down with the clamp on the rubber tube loosened, the level of the mercury can be adjusted very exactly. In the case of very delicate roots water could be used in the test-tube instead of mercury. In case a particular root shows any traumatropic bending due to the burnt cork (as may possibly be the case), a tiny glass cone, made from small glass tubing, may be sunk into the upper end of the cork.

Another method, which appears effective but not easy to apply, is that given by Sachs, in which the growing roots are made to push against tiny scale-pans connected over a pulley with small weights. A modification and improvement of this is proposed by Stone, in Botanical Gazette, **22**, 293. Sachs' experiment, in which the root grows downwards against the resistance of mercury, the resistance causing an upward bending just behind the turn, is well known but not easy to make work entirely satisfactorily. An easier way to apply the root to the mercury is the following: Take a block of wood 8 or 10 cm. long, 4 wide, and 3 thick; bore with an auger a large hole near one end 15 mm. deep, and fill it with mercury; pin the seed, previously germinated in sphagnum until the root is about 3 to 4 cm. long, firmly to the wood so that the root-tip lies upon the surface of the mercury; cover the whole with moist sphagnum and a bell-jar, and examine after a few hours. It is easy also by use of an inverted corked test-tube, and an inner smaller tube holding the seed, to make the growing root lift vertically the weight of the seed, and the tube in addition, proving in another way the tendency of the root to grow vertically downwards in preference to any other direction, even though a lateral growth would offer much less resistance.

From the preceding experiments it will readily be inferred that, while gravitation is all-important in determining the existence and the direction of geotropic growth curvatures, nevertheless it does not produce those effects by acting upon the plant as weight. It obviously acts simply as a stimulus to set in motion the geotropic growth processes and as a guide to direction. If this be true, it should be possible to produce upon the plant similar effects if the force of gravitation could be replaced by another force of similar intensity and as constant in direction. This can be accomplished by use of centrifugal force.

88. What is the effect upon the growth of geotropic parts if the force of gravitation is replaced by centrifugal force?

Experimentation by individual students upon this subject is hardly practicable, but you should become acquainted with it through observation of the demonstration experiment and the literature.

An excellent centrifugal apparatus driven by an electric battery is described by Arthur in the Botanical Gazette, **22**, 463, and is supplied with his apparatus. Another, very similar but driven by a hot-air motor, is described by Hansen in Flora, **84**, 352.

A simple form driven by a water-motor is described by Stevens (Botanical Gazette **20**, 89), and another simple form is figured by MacDougal. p. 56.[1] Probably one of the centrifuges used in Bacteriology, driven by the incandescent-light current, could readily be adapted to this use if supplied with a form of governor. A water-motor driven from a constant-pressure source would be best.

Your experiments have shown that stems and roots are geotropic, and the question arises as to what extent other organs are.

89. Do flowers show geotropism?

Answer by Experiment 53.

EXPERIMENT 53. This may be answered by observation of the behavior of young flowers in clusters made to develop in other than their natural position. Select a small plant with a developing cluster of (preferably zygomorphic) flowers, such as a potted Delphinium. Carefully bend over the stalk of the cluster and tie the latter as nearly upside down as possible; set it in darkness to make sure the resultant is not simply a light effect, and observe results.

90. Are leaves geotropic?

Answer by Experiment 54.

EXPERIMENT 54. Take a Tropæolum plant, and tie its stem to a stick so it cannot materially alter its position; place the pot on its side in entire darkness for twenty-four hours, and note the positions taken by the leaf-blades. Most other plants will behave similarly; try one with a pulvinus, such as a developing bean, and a "stemless" plant like an Oxalis.

91. What ecological phases of geotropism can you trace?

There is much simple and beautiful experimentation practicable upon dia-, lateral, and transverse geotropism, though time hardly admits of their further investigation here. The diageotropism of roots may be most beautifully illustrated by placing seeds upon the flat ends of the germinators used in the hydrotropism experiments (Experiment 58, Fig. 35). Also, it comes out clearly in seeds grown in the germination-boxes (Fig. 30). It is most instructive when, after the roots are of good size, the glass or other surface against which they grow is turned in the same plane through 45°. Very important ecologically, and easy to study, is the lateral geotropism of the stems of twining plants. To thoroughly test this, the plant should be placed with its support at various angles between the vertical and horizontal, and the critical angle below which it will not twine determined. The transverse geotropism of many root-stocks and trailing plants is to be noted.

92. In a paragraph express your idea, derived from consideration of all of your sources of information, of the exact nature of Geotropism, including (*a*) the factors concerned in the process, (*b*) its use to the plant, and (*c*) the reason why it is so extensively used.

B. *Heliotropism.*

It is a familiar fact that the green parts of most plants respond strongly to one-sided light, a phenomenon known as heliotropism (or phototropism). It is needful to examine its nature with more exactness.

93. How are growing stems, leaves, and roots influenced in direction by the direction of the light falling upon them?

Answer by Experiment 55.

EXPERIMENT 55. This may be tested by exposing germinating seedlings, growing in water, to one-sided light of constant direction. Prepare a tumbler-germinator like that used in Experiment 13 (Fig. 13), except that no black paper is to be used and the absorbing fabric must be near the top of the tumbler (to allow room for the roots) and must allow free access of light upon one side. On the fabric place 5 or 6 soaked mustard-seeds, fill to the proper height with tap-water; keep in darkness until stems are 3 to 4 cm. high, then cover with a hood in which is a hole 2 or 3 cm. in diameter at the height of the seeds. Place with the hole turned to strong light, and observe effect upon growth of the stems, leaves, and roots.

In general what is the difference in the direction taken by these three parts with respect to light direction?

When Geotropism and Heliotropism act together but in different directions upon a sensitive part, what is the result?

Answer by observation of Experiment 55.

For logical completeness, there should properly be tried here, as in the case of Geotropism, the correlative experiment of growing similar plants with light direction eliminated, either by its removal altogether or by its neutralization through use of a revolving clinostat. Since growth in darkness introduces unusual conditions and effects, the use of the clinostat is much the best. The clinostats already described under section 81 are of course ample for this purpose; the clock clinostats revolving horizontally will carry easily a five-inch pot, and very easily the germinator used in the above experiment.

For experiments upon heliotropism in adult shoots, Tropæolum, the Garden Nasturtium, is particularly good.

94. Do the reception of the heliotropic stimulus and the growth response necessarily occur at the same place?

Answer by Experiment 56.

Experiment 56. This may be answered if a plant can be found in which, when the growth zone is exposed to light but certain other parts are kept in darkness, no heliotropic movement occurs. In a small pot of earth filled to the brim (so that the edge of the pot cannot throw a shadow) sow a dozen oats, and place in darkness until they appear just above the surface. Prepare six very tiny cylindrical caps of tin-foil, 1 cm. long and just wide enough to fit over the tips of the young plants (they may be moulded on a large headless pin); quickly taking the pot from the darkness, slip the caps over six of the tips, thus covering them from light, and leave the others uncovered. Set the pot where it will receive very one-sided light and observe results.

95. What rays of the white light are most efficient in inducing heliotropic curvatures?

Answer by Experiment 57.

Experiment 57. This may be determined by noting towards what pure color of light heliotropically-sensitive plants most quickly turn. The colors may be supplied by screens of glass or gelatine, though the objections already mentioned (under their use in growth studies, page 108) apply to them for this purpose. Hence the pure-color chambers earlier described (page 108) should be used. The apparatus should be set vertically and a layer of compact sphagnum placed along the side (in this position the bottom) of the smaller pots, and on the moss a few oats. The whole is to be kept in darkness until the oats are 2 cm. high, when the color screens are to be put in place (the flasks are to be kept in position by gummed paper attached to their edges and to the felt paper of the glass cover), and exposed to a strong light for a few hours, when the comparative bending under the different colors may be determined, and measured.

Another class of light effects, distinct from the *directive* effects of heliotropism, are the nyctitropic, including most "sleep" movements. These are very easy to experiment with, using Clover, Oxalis, or Mimosa plants. Of still another character is the striking movement of chloroplasts towards or away from light, according to its intensity. Another class includes the light-protective movements shown by many Leguminosæ, and other plants.

C. *Hydrotropism.*

The tendency of roots to seek moist places is familiar and of much ecological importance.

96. To what extent are roots sensitive to moisture?

Answer by Experiment 58.

EXPERIMENT 58. This may be tested by so arranging roots growing geotropically downwards that they will be deflected if sensitive to a moist surface placed near them. Prepare a moisture-cylinder as follows. Take two thoroughly cleaned (and preferably sterilized) Zurich germinators; soak them a few minutes in water; then, holding them under water, run a broad rubber band around their junction. They may now be lifted from the water and they will remain full; a wire is placed around them as in Fig. 35 to prevent their coming apart and to form a support by which they may be suspended in a darkened bell-jar. Take now seeds of beans, corn, oats (or others in which the hypocotyl does not lengthen) which have been germinated in sphagnum to a cm. radicle, and attach them by slender rubber bands to the germinators, as shown in Fig. 35 with the radicles touching the moist surface. The cylinder is then to be suspended in a bell-jar, covered from the light, and placed in a favorable growth temperature. The bell-jar is not, however, to be tightly closed (for thus a saturated atmosphere would result in which the roots would grow vertically downwards): the stopper of the jar should be left out, and the rim raised a cm. or less from the table. Observe the growth of the roots and stems. What is the difference in the hydrotropism of roots and of stems?

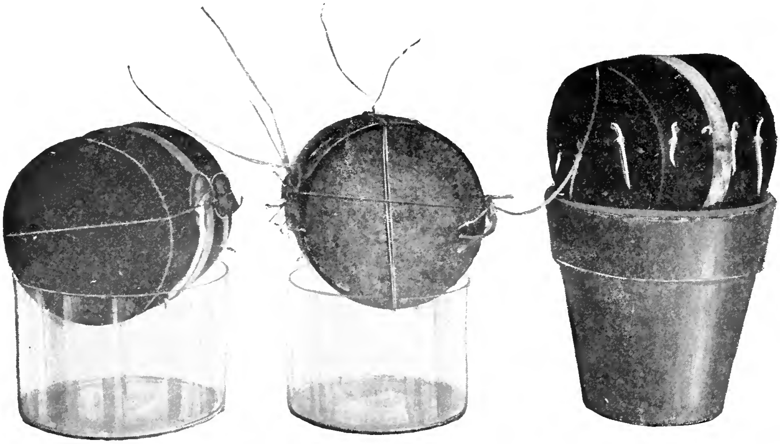

FIG. 35.—ARRANGEMENTS FOR THE STUDY OF HYDROTROPISM. One-third the true size.

Mustard-seeds may be used (Fig. 35, right) without preliminary germination; their mucilage attaches them sufficiently to the cylinder. Doubtless small flower-pots (with the holes stopped with corks) could be used as well as the germinators. It is not indispensable to use the rubber bands, for if the germinators are filled with sphagnum, as wet as it can be made, the apparatus works fairly well, though not so beautifully as when filled with water. The great value of this arrangement is that it gives a constantly increasing angle away from the geotropic direction, and hence allows the relative strength of the responses to the influences of hydrotropism and gravitation to be determined.*

This principle of hydrotropism may be demonstrated in several ways, of which the best known is that of Sachs, figured in all books, in which an inclined trough of wire gauze is used. It may also be shown by planting a germinating bean in a small flower-pot to which water is supplied by a strip of filter-paper running down between pot and earth and dipping into a water-reservoir outside; the height of the reservoir must be so adjusted that the soil is moistened slowly from the paper. Flower-pots may be utilized in various ways for demonstrating hydrotropism, advantage being taken of their porosity and their slopes. Another method, recommended by Arthur, is this: Take a funnel and cover its outer surface with filter-paper dipping into a water-vessel which supports it. Soak some large seeds and place them on sand filling the funnel; their hypocotyls are to project over the edge and they are covered by wet filter-paper. The roots will then follow the wet filter-paper along the slope of the funnel. I have effected the same end very beautifully by stopping the hole of a flower-pot, filling it with sphagnum and with water, and putting the germinating seeds (covering them with sphagnum) in the same way with hypocotyls pointing over the edge. The roots cling beautifully to the pot. In all these methods it is needful to adjust carefully the moisture in the air surrounding the hydrotropism-apparatus, which can be done by regulating the height the covering bell-jar is supported above the table; if the air is too dry, the roots wither; if too wet, they grow straight downwards.

D. *Thigmotropism.*

An influence to which plants are, very naturally, often sensitive, is contact, a property known as Thigmotropism.

97. What are the principal phenomena of response to contact in tendrils?

Answer by experiments (Experiment 59) devised by yourselves upon the tendrils of Melothria, Passiflora, Echinocystis, or other sensitive-tendriled plant.

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* Doubtless this arrangement could be used for investigation upon this subject. In some cases the roots after following the cylinder a definite and fairly constant distance turn abruptly downwards, thus giving a fairly constant "critical angle," which of course depends largely upon the degree of moisture in the chamber.

Observe the operation of the tendrils naturally clasping a support.

What is the rate of "revolution" of the tendrils?

98. What phenomena in response to various stimuli does the common Sensitive Plant show?

Answer by experiments (Experiment 60) devised by yourselves.

Can all of the responses to different stimuli thus shown be adaptive?

Do the responses continue actively if the stimulus is often repeated with equal intensity?

What is your explanation of the extreme sensitiveness to contact shown by this plant?

Another class of contact-effects are those shown by the irritable stamens of Barberry, Sparmannia, etc., and by the irritable stigmas of certain species of Mimulus, all very easy of experiment. Still another class is the growth of haustoria of parasites in contact with the host. The twining of some stems, e.g. Cuscuta, is in response to contact with the supporting host.

E. *Other Tropisms and Manifestations of Irritability.*

In addition to the above-mentioned four leading manifestations of Irritability, plants show many others, in which the responses to the stimuli are, as in the above cases, definite and adaptive. Some of the remaining manifestations are of great ecological importance. Most important of them is response to chemical substances (CHEMOTROPISM); in addition to its ordinary phases, best illustrated by roots and fungal hyphæ, there are special phases, such as sensitiveness to oxygen (AEROTROPISM), shown in Pollen-tubes, and, in another way, by the developing petioles of water-plants; yet another phase is the formation of galls and analogous structures; still another (partially chemotropic) is the development and ripening of fruits after fertilization. Important also is sensitiveness to heat (THERMOTROPISM), manifest in roots and stems, and with a special phase in which heat and cold give the stimulus to protective movements of leaves or stems. Others are sensitiveness to injurious contacts (TRAUMATROPISM), to the direction of water currents (RHEOTROPISM), to electrical currents, to some extent, (ELECTROTROPISM, GALVANOTROPISM). Sensitiveness to mechanical strains, resulting in growth of strengthening tissues, is widespread and ecologically immensely important. There are cases, too, in which the completion of one growth phase gives the stimulus to start the development of the next, as in the movement of some ripening fruits; this principle is important (perhaps extremely important) in ontogenetic development. And there are other manifestations of lesser importance.*

99. Prepare a classified table of the principal external influences which can be brought to bear as stimuli upon the protoplasm of plants, adding the probable place and method of reception of the stimulus, whether or not a response is known to take place, the mechanical basis of the response, its name, and its ecological significance.

100. Prepare a synoptical essay of not over 400 words upon Irritability.

CORRELATED TOPICS.

Analysis of the factors involved in irritable responses.

Significance of positive, negative, and lateral responses to the same stimulus.

Mechanical *vs.* other explanations of irritable responses.

Limits of irritability in relationship to heredity; cooperation of both in the development of individual form. Sachs' theory of development.

---

* This subject of Irritability, though vastly important, is not treated here more fully for three reasons: (1) enough has been done to demonstrate its fundamental character and its principal manifestations; (2) time will not permit its greater extension in a one-year course without forcing out other topics; and (3) the further investigation of the subject belongs rather to ecology than to pure physiology. There is, however, a great deal of simple valuable and practicable experimentation upon the above and related topics, to which Darwin and Acton and Detmer-Moor form very good guides. Such experimentation may mostly be carried on out of doors in the summer, and it could very well be made the theme for holiday summer work.

Relations of intensity of stimulus to amount of response; Weber's law.

Tonic, or formative, *vs.* directive effects.

Relation of responses to respiration and growth.

Correlations; induction effects; acclimatization to stimuli.

After-effects; reaction times; latent periods; rigors; rhythms.

Perceptive and motor regions in different organs; transmission of stimuli.

Mechanisms which effect responses.

LITERATURE.

There is an immense literature on Irritability, which will unquestionably be summarized in Vol. II of Pfeffer's Physiologie. Until the appearance of that work the subject may be traced through the following:

Pfeffer, W. Die Reizbarkheit der Pflanzen. Leipzig, 1893.

Czapek, F. Writings upon Geotropism in recent volumes of Jahrbücher für wissenschaftliche Botanik.

Noll, F. Ueber Geotropismus. Jahrbücher für wiss. Botanik, **34**, 457.

Davenport, C. B. Comparative Morphology, Vol. II.

Pfeffer, W. Geotropic Sensitiveness of the Root-tip. Annals of Botany, **8**, 317.

Spalding, V. M. The Traumatropic Curvature of Roots. Annals of Botany, **8**, 423.

Newcombe, L. C. The Regulatory Formation of Mechanical Tissue. Botanical Gazette, **20**, 441.

Juel, H. O. Untersuchungen über Rheotropismus. Jahrbücher für wissenschaftliche Botanik, **34**, 507.

Jost, L. Beiträge zur Kenntniss der nyctitropischen Bewegungen. Jahrbücher für wiss. Botanik, **31**, 345.

MacDougal, D. T. The Curvature of Roots. Botanical Gazette, **23**, 307.

—— Mechanisms of Movement and Transmission of Stimuli in Mimosa and other Sensitive Plants. Botanical Gazette, **22**, 293.

Section 5. Locomotion.

Section 6. Protection.

Both of these topics are almost exclusively ecological, with but a slight basis in pure Physiology, and hence do not come within the scope of this course. They are to be pursued in the field, and are proper subjects for investigation through the summer.

ADDENDA.

IN the time that has elapsed between the completion of the manuscript and the printing of this book, some improvements in the foregoing methods have been effected, as follows.

1 (page 66). The chief drawback to the use of the Pfeffer cell is the uncertainty of its working. However, the porous cups and filter bougies, having a straight tube sealed into them with sealing-wax, always work well with me up to a pressure of somewhat over one atmosphere, above which the molasses (which I generally use) escapes through the cup into the outer vessel. It is, by the way, somewhat better in preparing the cups to place them in the copper chloride or sulphate solution and exhaust thoroughly under an air-pump. Also, it is very easy to make the diffusion-shells semi-permeable by thoroughly soaking and warming them in water until all air is removed, when they are to be filled with the potassium ferrocyanide solution and placed overnight in copper sulphate solution. They may then be filled with molasses and will always quickly give a pressure of over an atmosphere, after which the membranes appear to burst, for fine jets may be seen issuing from the sides of the shell. The shell thus prepared will raise a column of water three or four times as high as when unprepared.

Perhaps this subject is not worth the trouble given to it; but its value lies in the excellence of its illustration that the work of the plant can be done by ordinary physical forces.

2 (page 68). For example, the extreme vapor tension for 15° C. is 12.70 mm. of mercury, that is, about $\frac{1}{60}$ of an atmosphere; for 20° C. it is 17.39 mm., or about $\frac{1}{43}$ of an atmosphere. The error in the gauges will of course be in the direction of a lesser result; that is, owing to the vapor tension, the pressure is really greater than it appears to be from the level of the liquid.

It may, however, be exactly calculated from the following table, in which the pressure is expressed in millimeters of mercury for each degree of temperature. To apply it, one of course pays no attention to it at the reading made when the tube is first sealed, but in all subsequent readings one first calculates the pressure in fractions of atmospheres (or else reduces it to millimeters of mercury) as if no vapor-tension were present, and then adds to this result the pressure obtained for the given temperature from the table, either reduced to fractions of an atmosphere or in millimeters of mercury.

Temp. C.	mm.	Temp. C.	mm.	Temp. C.	mm.	Temp. C.	mm.
10	9.17	16	13.54	22	19.66	28	28.10
11	9.79	17	14.42	23	20.89	29	29.78
12	10.46	18	15.36	24	22.18	30	31.55
13	11.16	19	16.35	25	23.55	31	33.41
14	11.91	20	17.39	26	24.99	32	35.36
15	12.70	21	18.50	27	26.51	33	37.41

It is usually not difficult to bring the gauge for the final reading into approximately the same temperature as that at which the first was made; but if this cannot be accomplished it is easy to make the correction for it by the calculation that the gas in the tube expands or contracts $\frac{1}{273}$ of its volume for each degree of temperature C.

In experiments involving pressures, wherever there is an outside free surface of mercury or water, a correction should be applied for barometric changes, but here again the possible error is hardly great enough to materially affect the comparatively gross measurements in these experiments. But the fact that all these errors exist, their nature, and the method of correction should be illustrated.

3 (page 69). Not only with these, but with all gauges, it is necessary to insure that the gauge can register a maximum pressure within the amount of movement which the plant has available for showing the pressure. Thus, if water is being given off from the plant at a pressure of one atmosphere, but the quantity is only 20 cc., and a comparatively large open gauge is used, obviously the 20 cc. of water would not be sufficient in quantity to push the mercury 760 mm. high, as it must to register the pressure of an atmosphere. Hence the gauges should be of such a sort that several atmospheres can be registered by a much smaller quantity than 20 cc. This applies also to the use of spring-balances for registering pressures of swelling seeds, etc.

4 (page 79). After somewhat extended quantitative tests of various methods of enclosing the soil to prevent evaporation, I have concluded that the two methods possessing the greatest advantages are the following: A zinc pot is made of a size to cover the flower-pot; it costs but little and can be used for years. The top is strengthened by a wire, just below which a groove is made. The rubber sheeting, with a hole in its center, made by a large cork-borer, is then used as previously described, to connect plant and zinc-pot, and it is fastened to the latter by wire

twisted tight, or by a very strong tightly stretched rubber band, which is prevented by the groove from slipping down the cover. The outer pot could better be of aluminum. Another method, which weighing tests have shown to be the best of all for preventing evaporation from the soil (for the rubber is not a perfect preventive), is this: Remove a plant from its pot and plant in a glass jar as previously described; the earth should not come to within a half-inch or more of the top. Wrap the stem of the plant from just below the ground to an inch above it with two or three turns of rubber, and pour over the soil successive layers of melted paraffine heated barely hot enough to melt it. After it has hardened it will shrink away from the jar, but may here be remelted by a hot iron. This method does not injure the plant, and seems a perfect preventive of loss of water otherwise than through the shoot. It is well to cover the jar with black paper to shield the new young roots from intense light. Amongst efficient balances for use in transpiration, that figured in Pfeffer-Ewart, page 241, should be noted.

5 (page 81). A decided improvement in the potometer is the following: Instead of turning the tube down at one end (Fig. 15 on the right) it should be turned upward and connected with an upright tube like that beside the shoot. The water placed in this tube supplies some pressure to the shoot and appears to make it work more evenly than when the water is drawn from the vial below the tube. Bubbles of air can be admitted by a small hole bored through the tube (by the method given on page 43), or by a break in the tube, kept covered by a piece of rubber tubing which can be slipped aside to admit the air. The tube must be perfectly clean inside, or the bubble of air will move unevenly.

6 (page 83). An excellent method of applying the cobalt paper is the following: With the largest cork-borer cut out rings from two good corks, so that they will be 5 mm. high and thick. Glue pieces of long-piled velvet or plush upon one face of each, and when dry trim it down and remove with the cork-borer, so that it finally covers only the ring of cork. Over the other face of the ring glue a cover-glass of proper size. Make from elastic wire a spring-loop like those of test-tube holders, and push the two ends into the corks. The two corks should now be held with their velvet faces against one another by the spring of the wire. A piece of the cobalt paper is then placed in each one, the apparatus is sprung open, and the corks are allowed to rest on the two surfaces of the leaf: the velvet makes closed chambers against even the inequalities of the leaf, and the change of color in the cobalt paper may be clearly followed through the cover-glasses. A new form of hygrometer, especially for use in determining loss of water from stomata, is described by MacDougal in Torreya, I. 16.

7 (page 89). I have obtained excellent results by the use of the above-mentioned colored liquids placed in small square bottles glued side

by side. To enclose areas of the leaf so that only a single color will reach them, rectangular openings were cut in a piece of velvet to match the smooth faces of the bottles; this was then glued to them in such a way that the joints between the bottles were covered by the velvet and the leaf was exposed only under the flat face of each bottle. The other side of the leaf was covered by black velvet glued to a strip of glass. Under this arrangement the iodine test showed that in a day nearly as much starch was made under the red screen as under the white one (distilled water), while a very much less, but still appreciable, quantity was made under the blue, and none at all under the green. To prevent the corks being forced from the bottles by expansion of the liquids under heat, a thread between cork and neck connected the air-space over the liquids with the air outside.

8 (page 94). For strict accuracy, allowance must be made in this apparatus, not only for temperature, but also for vapor-tension and barometric pressure, concerning which corrections see note 2 above.

9 (page 98). There are other methods of subjecting seeds to growth conditions without oxygen, such as surrounding them by a neutral gas (hydrogen), but the following gives perfect results: Take two of the slenderest test-tubes, and, heating them near the neck in the flame, draw them out until the neck is but 2 or 3 mm. in diameter; when cool slip into each two or three soaked oats from which the glumes have been removed. Into one of them put a bored rubber stopper holding a glass tube, to which attach a tube from an air-pump, such as the Chapman pump, and exhaust thoroughly; while the exhaust is still on heat the narrow neck with a bunsen flame until it seals itself entirely. Then treat the other similarly except that, before sealing, the air is to be admitted (the exhaust is used on the latter also in order to be sure that the ultimate difference may not be due to something connected with use of the exhaust). The two tubes are then put aside in a favorable growth-temperature. Instead of the test-tubes ordinary glass tubing sealed at one end and drawn to a narrow neck near the other may be used.

Another very ingenious method, which I have repeated with entire success, is that given by Murbach in School Science, I. 25. It is, however, much more effective to use a control experiment than simply to test the vitality of the seeds later, as Murbach recommends. The control would be treated in every way like the principal tube except that it should be allowed to cool, and hence readmit air, before being sealed. The perfection of the vacuum in such tubes may subsequently be tested by holding the sealed end under water and cracking it off with nippers, when the height to which the water rises in the tube will give a measure of the vacuum, which can be made nearly perfect by this method. Oats give excellent results. One must be careful not to explode the tube in sealing it, which may be guarded against by using the same flame to boil and to seal the tube. Murbach's third method seems faulty both in that it is impossible to obtain pure carbon dioxide from the lungs, and also because carbon dioxide is not a neutral gas but exerts active poisonous effects upon the living seeds.

10 (page 110). Far better for this purpose is a differential thermostat which I have had made, but too late for full description in this work. It consists essentially of a strip of thick copper having at each end large cold- and hot-water boxes, and ten chambers large enough to hold each a Zurich germinator along its length. When cold water from a tap is kept circulating through the cold box, and the water in the other is heated by a bunsen flame controlled by a Reichert regulator, the ten intermediate chambers keep a perfectly even gradation of intermediate temperatures, which may be held constant for days together, and which may be made to differ in greater or lesser amount by altering the heat from the burner and by changing the rapidity of circulation of the cold water. In this instrument it is possible to determine very accurately the minimum, optimum, and maximum temperatures for germination of seeds and early growth of the seedlings, and moreover several kinds can be observed at once. The chambers communicate by small openings so that water to supply the germinators stands at the same height in all of them, and it is supplied at the desired height from a self-regulating apparatus such as is figured earlier in Fig. 27, page 109.

Page 41. A method of making a gas-tight joint with a living plant is given by Arthur and MacDougal, in "Living Plants and their Properties" (New York, Baker and Taylor, 1898), 126.

Page 51. Distilled water is never to be used for mounting living cells, since endosmotic action becomes active and does them more or less injury.

Page 76. A simple dynamometer adapted to measure the power exerted by swelling seeds, etc., and which is to be used with the precautions mentioned in Note 3 above, is described by Richards in Torreya, I. 8.

Page 101. Add to the Literature, Arthur and MacDougal, "Living Plants and their Properties," especially Chapters VIII, IX, and X.

Page 102. The auxanometer should be used to determine the grand period of growth in some such structure as a flower-scape. It is instructive to compare the growth in this way of two similar parts, one in light and the other in darkness.

Page 134. Add to the Literature, Arthur and MacDougal, "Living Plants and their Properties," especially Chapters I, II, and IV.

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