A LABORATORY COURSE

IN

PLANT PHYSIOLOGY

SECOND EDITION

EXTENDED TO FORM A HANDBOOK OF EXPERIMENTATION FOR EDUCATIONAL USE

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PREFACE.

This book has a threefold purpose. First, it aims to lead students through a good laboratory course in Plant Physiology. Second, it seeks to provide a handbook of information upon all phases of Plant Physiology having any educational interest. Third, I venture to hope that it may find service as a guide to self-education by ambitious teachers or students, who, unable to obtain regular instruction, yet wish to advance themselves in this attractive and important subject. To meet these ends I have tried to bring together the best of that which is already known in its field, and to add thereto some materials derived from new studies. The book is not a compendium of physiological knowledge, nor yet, except incidentally, a handbook of investigation; but it is a guide to the acquisition of a physiological education. It is designed as a contribution to educational economy, and as such I wish it to be judged.

If the book is found to have any special merit in its particular field, this will no doubt consist in its practicability, for, with but few and mostly obvious exceptions, everything in it has been tested repeatedly by my students and myself. In this, as in some other respects, however, the work is uneven, and some parts have been worked out far more carefully than others. The faults I hope in the future to correct and the deficiencies to supply. Nobody can be more conscious than I of its imperfections, and of how much remains to be done to put our physiological education upon a satisfactory basis. But at least we may feel assured that we are on the right road, and have made a goodly start.

Although the general aim, and therefore the principal title, of this book remain the same as in the first edition, it has been entirely rewritten and much extended. The chief differences, aside from improvement in details, consist in two things: First, there is much greater insistence upon mechanical neatness in experimentation, upon effective exposition, and upon scientific logic. Second, the emphasis is thrown, for advanced or college work, not upon qualitative results obtained by students from apparatus of their own making, but upon quantitative results obtained from practically accurate, or normal, apparatus manufactured expressly for its particular work. The reasons for this change will be made plain a few pages later. I wish here simply to emphasize its presence and its importance.

The first edition of the book had the advantage of criticism in manuscript by Professor C. R. BARNES, and much that he suggested is retained in this edition. I have had, as before, the constant co-operation of Mr. E. J. CANNING, the skilled Head Gardener of Smith College, in the effort to find the best materials for educational work. I have received, also, much aid from many of my loyal students, some of whom are mentioned by name in the following pages; but I am especially indebted to Miss SOPHIA ECKERSON, whose aid and criticism have been constant and valuable. To the BAUSCH and LOMB Optical Company I owe the use of the cuts illustrating my normal apparatus made by them, and they have permitted me also to copy freely from their copyrighted descriptive catalogue of this apparatus. To those who have thus co-operated in making this work more serviceable, I wish to express the grateful thanks not only of myself, but of all who may find the book of use.

WILLIAM F. GANONG.

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PART I.

ON THE EDUCATIONAL PLACE, METHODS OF STUDY, AND NECESSARY EQUIPMENT OF PLANT PHYSIOLOGY.

1. THE PLACE OF PLANT PHYSIOLOGY IN BOTANICAL EDUCATION.

THE most striking feature of recent educational progress in Botany is the increased attention paid to Plant Physiology; and there is every indication that this advance is permanent, and destined to yet greater enlargement. For this movement there are several good reasons. *First*, there is a general tendency in education towards greater emphasis upon vital or dynamical phenomena. *Second*, there is everywhere a growing interest in all matters pertaining to life, constituting as they do, useful and illuminating knowledge. *Third*, experience seems to be showing that the physiological groundwork of a biological education may best be obtained in part from plants, most of whose physiological processes are identical with those of animals, while much more accessible to experimental study. *Fourth*, physiology is the indispensable basis for advance in that ever-alluring but still rather barren branch of itself, Ecology, which differs (or ought to differ) from pure Physiology only in this,—that it deals with large and conspicuous phenomena. *Fifth*, the possibility of making discoveries of far-reaching scientific importance, or of great practical value to mankind, is vastly greater in physiology than in any other field of botanical investigation. *Sixth*, all the modern problems of plant culture,—the defeat of disease, the improvement of existent forms, the development of new qualities or races,—are coming more and more to be approached, even from the purely practical side, through the exact knowledge and the characteristic method of Plant Physiology. Hence the subject has assumed great prominence in all agricultural and horticultural institutions,—Departments, Experiment Stations, Colleges,—and even in the trial grounds of practical growers of plants; and there is well-nigh universal belief that great results are to be expected in this direction in the future. Whether for one, several, or all of these reasons, every educational institution aiming to put its students into touch with contemporary progress has established, or must establish, competent instruction in Plant Physiology. And the subject will rise still higher in general estimation as it advances to a loftier plane of scientific efficiency.

It is fitting, now, to consider the place the subject should have in the educational curriculum.

In an ideal plan of study the student would first come into contact with Plant Physiology, though not by that name, in his nature study in the lower schools. Here he should learn, along with the salient facts about the common plants around him, and simply as fact, without regard to method or system, those great physiological truths which mean so much for an understanding of the circumambient world, such as these:

That roots absorb water and minerals and pass them up to the stems.

That stems conduct water with dissolved minerals from roots to leaves, where the water is evaporated, and that they spread the leaves out into the light.

That green leaves in light absorb carbon dioxide from the air, and, combining this with water and minerals from the soil, manufacture their food, which food nourishes not only them, but also all animals.

That all living plants need oxygen precisely as animals do, and, like them, give off carbon dioxide, and it is only in green leaves in light, that a reverse process also occurs.

That the growth of plants is promoted by warmth, ample oxygen, sufficient moisture, and, sometimes, by darkness.

That growing parts have the power to turn towards, across, or away from light, water, or the up-and-down direction according as may be best for the performance of the functions of the parts.

The experimenting should be all of the simplest sort, with apparatus put together by the pupil himself; for the child is very self-centered, and can best be taught through appeal to his self-importance. Later in this book, under the appropriate topics, and usually in connection with "make-shift" apparatus, I give such suggestions as I can upon this teaching.

The student should next meet with the subject in his elementary course in the science of Botany in the high school or the first year in college. Here, with good appliances used by himself, or at least made familiar by thorough demonstration, and with attention to scientific method, he should study the fundamental topics of scientific Plant Physiology. His study will necessarily be qualitative rather than quantitative, and it should deal with the topics not as isolated facts, but in connection with the structures and habits which they help to explain, and as integral parts of that organized whole which a course in a science should represent even under elementary treatment. The precise physiological topics which should be considered in an elementary course in Botany have been much discussed by teachers, and there is room for difference of opinion upon the subject. But the recommendation thereon having the highest educational standing is contained in a Report,* originally formulated by a committee of botanical teachers and endorsed by the Botanical Society of America, now used as a basis for its work by the College Entrance Examination Board. The topics, which are not intended to be studied all together by themselves, but rather here and there in the course along with the structures with which they are associated and which they help to explain, are the following, those in italics being intended to be studied experimentally:

Rôle of water in the plant: *Absorption (osmosis); path of transfer; transpiration; turgidity and its mechanical value; plasmolysis.*

Photosynthesis: *Dependence of starch formation upon chlorophyl, light, and carbon dioxide; evolution of oxygen;* observation of starch grains.

Respiration: *Necessity for oxygen in growth; evolution of carbon dioxide.*

Digestion: *Digestion of starch with diastase,* and its rôle in translocation of foods.

Irritability: *Geotropism, heliotropism,* and *hydrotropism.*

Growth: *Localization in higher plants; amount in elongating stems; relationships to temperature.*

Fertilization: Sexual and vegetative reproduction.

In the proper places later in this book, usually under the head of "demonstration" or "adapted" apparatus, I shall

* The fourth edition of this somewhat important educational document is to be published in the School Review in the autumn of 1908.

contribute such directions and suggestions as I have been able to develop in my own experience for the teaching of an elementary course.

We consider finally the place which a course in Plant Physiology, treated by itself as a unified subject, should hold in the college curriculum. Theoretically it would seem best to introduce it as early as possible, even before the study of structure upon which it throws a flood of light. But here, as so often in teaching, that which is fair in theory plays false in practice. Many of the facts and phenomena with which Physiology deals are of so abstract and unfamiliar a sort that the student must have a considerable foundation in concrete fact, scientific training, and mental maturity, before he is prepared to profit fully by the subject. Moreover a later position for this study allows the student more time to obtain that training in Chemistry and Physics, in Zoölogy and Meteorology, and in German, which form such desirable, if not essential, preliminaries to Physiology. From all points of view it seems best that the biogenetic law should be followed, and that the subject should come latest in the student's training, as it has come latest in the development of the science. The undergraduate course in Botany which seems to me to offer the optimum of advantage to the student would follow somewhat the following plan:

The First Year (whether in high school or college). 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. My experience with such a course is embodied in my book, "The Teaching Botanist" (New York, The Macmillan Co.).

The Second Year. Course in Morphology, tracing the structures, relationships, and adaptations of the Groups from the lowest Algæ to the highest Phanerogams.

The Third Year. Course in Cellular Anatomy, with Cytology and Embryology.

The Fourth Year. A Course (or Practicum) in Experimental Plant Physiology, upon some such plan as is developed in this book.

After such a course the student should be prepared to undertake university or other research work. This arrangement seems to me best for colleges in Arts. For those in Agriculture it might be better to condense into one the above-described courses of the second and third years, so that Physiology may be reached earlier.

It may be objected that such a course gives too much routine work, and that after two, or at most three, years of regular courses the student had better be given some original problem in which his powers of investigation may be cultivated. Aside from the great inherent difficulty of securing investigation from undergraduates, with all the demands upon their time and attention, 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 of the courses of the four years will be, from the student's point of view, 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 every 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 proposed course is too inelastic, too mechanical, for the good of advanced students, and especially for those of marked originality. 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; while it is surely desirable that all students should be trained in the substance of the accumulated knowledge of the race, and in approved methods of manipulation, before being set to find out or to do something new. It is entirely possible for the framework or plan of a course to be rigid, while its clothing of details is plastic under the master's hand. Upon the whole 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.

2. TEACHING AND LEARNING PLANT PHYSIOLOGY.

It is altogether probable, as it is certainly most desirable, that wherever a course in Plant Physiology is taken up, it will be under the direction of one who is both a trained botanist and a skilled teacher; and it is equally likely that any students electing it will already have acquired some proficiency in scientific ways of working and thinking. Hence it may seem superfluous to volunteer advice either upon teaching or upon learning Plant Physiology. But discussion and comparison contribute to progress, and therefore I shall venture to summarize the characteristics which seem to me to mark good teaching and good learning in Physiology; and I shall add 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 it 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 as well as to know. He utilizes the good and pleasurable instincts in his students, their curiosity, their pleasure in competition, their artistic sense, their individual talent, and their ambition. At each new step in their work he recalls to them that which they already know, and thus develops a vantage-point in the known from which to lead their sorties into the unknown. He tries always to create a demand for truth before he provides the supply. He habitually illustrates proper inductive procedure; keeps prominent the conception of the conservation of matter and of energy; 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.

There is one accompaniment of good scientific teaching which fully deserves the great emphasis it often receives, and that is the doing of some investigation by the teacher. The dominant idea in all scientific teaching, from the kindergarten to the university, should be the inculcation of the spirit of investigation,—the instinct to attack new problems with a determination to solve them by one's own efforts. Only the teacher who feels this spirit can impart it, and only he can feel it who is ever exercising it. For university and most college work this is now viewed as axiomatic, but it is true in principle for all grades of teaching. For most teachers, however, as well those in many colleges as in most schools, the limitations of training or equipment, or the demands of the teaching, which itself is properly entitled to the teacher's first and best efforts, effectually prevent any investigation of the abstract scientific sort. It is very fortunate, therefore, for the teacher of this subject, that there lies open an attractive and profitable field of investigation in the educational phases of the subject itself. As the following pages will show, some of our physiological experiments, appliances, and methods are educationally satisfactory, being accurate, practicable, and profitable; but others, including still the majority, are imperfect and susceptible of great improvement. Again, in only a few cases do we know which of the accessible plants are best for the study of a particular subject, or in just what way they should be treated to yield the best results, or how much, quantitatively, may be expected of them; and there is much to be done in this direction. Further, the extreme specialization of science, and the consequent inaccessibility to general students of most of its later results, are giving a positive value to good comprehensive expositions of scientific topics, such expositions as combine literary excellence with pedagogical force and scientific accuracy; and the presentation of such expositions in the educational journals constitutes also a form of serviceable educational investigation. For success in any of these lines the teacher must carry on actual careful prolonged experimentation and study, but in the doing of any of them there is great reward.

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, and seeks to acquire their open, judicial, evidential habit of mind, which alone can lead to scientific success. He acquires 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 not until 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. He develops such self-confidence that he expects all his work to succeed, and is surprised when it does not. He is perfectly honest with all his experiments, never views them through preconceived expectations, never glosses irregularities, and never ignores exceptions, not only because these things are dishonest and unscientific, but also because he may thereby miss one of those clues which, as experience has shown, often lead to the greater discoveries. 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. He takes steps to ensure that his work may be done in physical comfort, and he does it with a feeling and an air of academic calm and leisure.

A college course in Plant Physiology should be dominated by a spirit of precision, quantation, and logic. The aim in the course should be as it is in the science,—to express physiological phenomena in exact figures whose correctness is guaranteed by accurate scientific logic. For this purpose it is requisite that the student shall have the use of good laboratory and greenhouse facilities, ample materials, and efficient tools,—that is, apparatus. All these I shall describe a little later, but I wish to emphasize the need for them here, because there has grown up among us an idea that such facilities are not only unnecessary for good work, but even are of less worth than the simple arrangements which the student can improvise or adapt for himself from easily procurable materials. The argument is, that such adapting inculcates ingenuity, facility in manipulation, and self-reliance. I have myself in the past been an advocate of this idea, but a longer experience as a teacher has shown me that it is fallacious, and that the method is educationally wasteful if not pernicious. For one thing the student's time, attention, and energy are so largely absorbed by the preparation of the apparatus that he has little of either remaining for observation of the phenomena of the plant, and for another the crudeness of tools and consequent looseness of results inculcate a wholly wrong ideal and habit of scientific work. And as to skill in manipulation, that is best acquired by systematic practice, at first under guidance, in the essential processes preliminary to experimentation, while the training in ingenuity and self-reliance depend less upon the method than upon the spirit of the course. There is nothing peculiar to Plant Physiology whereby in it alone of all the sciences it is better to do imperfect work with self-made tools than to do exact work with good tools made expressly for the purpose. I speak now of college work in an organized course in the subject; in the demonstration work of an elementary general course the simpler appliances showing qualitative results are more in place, while in lower grades the arrangements improvised and made up by the children are actually the best, for to them the things which they do for themselves are of more account than all the wisdom of the sages.

Correlated with goodness of facilities and tools goes proper neatness in their use. The experiment house should be kept in the perfection of condition, the more especially as the nature of the material tempts to untidiness and the present education of youth predisposes to carelessness. A cleanliness habit should prevail, leading among other things, to the cleaning of all appliances before they are put away, and a cleaning again before they are used. Plants under experiment should have clean pots with saucers, and should be kept well watered and well groomed, and should never be allowed to exhibit the distress of neglect. Apparatus should be set up in a neat and workmanlike manner, and should be placed exactly in the middle or focus of the table, with a clean margin about; and nowhere should loose ends, makeshift devices, unsymmetrical arrangements, forgotten tools, dust or debris appear. Indeed it is not too much to expect that all experimentation should be done in a manner distinctly artistic, with attention to all those details which go to make up a pleasing effect. These features have not, perhaps, so much value in themselves as they have in their reflex effect upon the workers. A slovenly experiment house is an advertisement of slovenly minds in charge, and a temptation to slovenly work, while an artistically kept house reflects a careful spirit and encourages to precise work. Care, attention to detail, neatness, a large simplicity,—these are scientific.

Plant Physiology is distinctively an experimental science, and hence necessitates a knowledge of the nature and the logic of experimentation, together with a habit of the proper use thereof.

A really good experimenter is born, not made; for there is a sort of experimental instinct, which includes a combination of 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 be so planned as to call for a simple answer, and of course should be as little complicated and as little damaging to the plant 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 this 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, and always best, to try at the same time a parallel experiment in which a similar plant is placed under precisely the same external and experimental 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 a parallel experiment is called a *control*, and an impulse to control experimenting is an essential part of the experimental habit. It is through lack of it that our elementary botanical text-books are disfigured by descriptions of some experiments which are scientifically illogical, and which only by accident give correct results, a subject on which I shall comment at the proper places in this book.*

Control experimenting tends to neutralize some of the grossest of the sources of error which beset all scientific investigation; but there remain many others whose detection and elimination

*I have also discussed it, somewhat fully, in School Science, 6, 1906, 297.

should be the constant care of the student. The more prominent of these may be classified thus:

1. *Errors of the Instrument.* Some of these are so crass as to be obvious, such as crudeness, shakiness or leakiness due to poor mechanical construction (very apt to characterize makeshift or even adapted apparatus), and presence of dirt, or of chemicals unremoved from earlier experiments. More serious are errors of standardization, due either to the carelessness of the maker or his desire to sell the instrument cheaply (as prominently shown in cheap thermometers); the remedy is to buy good articles from makers with a reputation to sustain, and also and especially, to test the standardization by comparison with instruments of known accuracy. A still more serious, because so insidious, phase of this error comes from spontaneous change in the standardization. To some extent thermometers, especially when newly made, are liable to this. In another way, small weights are very susceptible to alteration through adherent grease, etc. Self-recording meteorological instruments, such as thermographs and hygrographs, are very liable to work themselves out of true, and should often be standardized by comparison with instruments of known accuracy. Errors, of obscure cause, may intrude in control experimenting from the use of dishes or other articles differing merely in size, shape, or color, and these should always be chosen exactly alike even when there is no conceivable reason why differences of this sort should affect the result.

2. *Errors of the Surroundings.* These are connected largely with meteorological changes, which are incessant. Thus, readings made at different temperatures may be vitiated by the expansion or contraction of metal, or even glass, parts of the instrument, and especially of mercury where that is employed. Readings made at different humidities may involve a serious error from the hygroscopic lengthening or shortening of papers or of threads, especially if the latter are so used that their alteration may be magnified. This happens commonly with the cords of auxanometers, where the error may become so great as actually

to record the reverse of the real facts. Readings of the volumes of gases gauged by the surfaces of liquids are liable to several errors. When the liquid communicates with the atmosphere, error is introduced by changes of barometric pressure, or by vapor tension, which alters greatly with temperature, or by variations of temperature which affect the gas-volumes. Capillarity is another disturbing factor where liquids are concerned. Some of these errors are so well known that they can be calculated from tables prepared for the purpose, of which those important to Plant Physiology will be found in Part III of this book; but others can be met only by special precautions for each case. In their relations to external conditions plants are so complexly sensitive, that it is essential, in control experimenting, to ensure that they shall be exposed to surroundings precisely the same even to minutiæ.

3. *Errors of the Organism.* The chief error from this source arises from the innate variation between any two individuals, no matter how closely related. For this reason, where two plant parts are required, as in control experimenting, these should be from the same stock, or as closely akin as possible. But it is much better to use two shoots or roots of the same plant, better yet to use two leaves, or other parts, of the same age on one shoot, and best of all to use two corresponding parts of the same leaf or other part, thus practically eliminating the individual error. It is for this reason that so many of the experiments recommended in this book are arranged, even at the expense of considerable complexity, to use for experiment and control two corresponding parts of the same structure. Where, however, it is impossible to avoid using separate plants, it is best to employ a considerable number, preferably at least ten, when, by taking the mean of the results of all, the individual error is minimized if not eliminated. Difference of age of parts is especially liable to introduce serious error, but mechanical injury, though serious, is, owing to the low degree of division of labor among plant cells, less serious than is commonly thought.

4. *Errors of the Person.* Of these I need hardly include

the obvious ones arising from carelessness, clumsiness, or other personal incapacity, for the student of such characteristics will stop short of this course. But of the legitimate personal errors there are several, against which the student should be forever and consciously on guard. Thus, in matters involving observational judgment, as when spaces of time or distance must be read by estimation, the personal equation, a well-known source of error, intrudes. It can be met only by ascertaining one's own tendency, whether too fast or too slow, and by constant comparison with the averages obtained from many others. A common and concrete personal error lies in a tendency to misread fractional parts, as of degrees, weights, etc., when hurried, the more especially if the scale must be read inverted. The remedy is this, never to be hurried, and also to cultivate the habit of always reading such figures twice, if possible in a reverse direction or in a different frame of observational mind. And especially the habit should be formed of concentrating the whole attention upon that one single thing for the moment, with a conscious effort and resolve to read correctly. When the results of any given study are mathematical, there are mathematical methods, more or less easy of application, for determining the probable error. There is, however, a simpler way which, for most student work, is practically effective, namely, to compare one's own results with the mean of those obtained by other students working at the same time upon the same problem. To establish such a mean it is needful that all should work by the same methods and express their results in the same units.

5. *Errors of the Mind.* These arise from the fact that the mind, our only tool for the investigation of the abstract and the unknown, is an excessively poor one for the purpose, because it was developed in adaptation to efficiency in a totally different direction, namely, material success in a struggle with a world of concrete fact. Hence we have a sense of impotence in the presence of the unknown which makes us willing to rest content with mysterious, metaphysical, or even complexly verbal explanations, without demanding clear definition and evidence

of correctness. This spirit is in direct contradiction to that of reliance upon the conservation of energy and of matter, though the latter is a chief mark of the scientific mind. Again, we have the primitive tendency to link together by some mysterious bond of cause and effect any two striking things which come to occupy our attention simultaneously, though in fact they may be only accidentally or coincidentally connected. Again, the defensive instinct leads us to interpret everything in the direction of self-justification or self-magnification, to warp observation into support of preconceived ideas, and to magnify the importance of any new thing discovered by ourselves to the disparagement of the old as expounded by others. Again, we have the old habit of following blindly our constituted leaders, whence arises altogether too great reliance upon authority, especially of the eminent. And we have always a primitive delight in wonders, and corresponding willingness to believe in them, and an ever-present predilection for pleasing fiction over commonplace fact. All these peculiarities of the mind are quite in harmony with its use in the struggle for material existence, but they are frailties when it is applied to scientific investigation. They tend ever to warp the judgment from the objective towards the subjective, and against them the student must learn to be ever upon guard. This applies to the use of mind as it is, and is wholly apart from the possibility that some of the phenomena of Nature may be of a kind which we have neither the senses to perceive nor the mental equipment to apprehend.

Such are the more important working principles and precautions needed in Plant Physiology. If the student cares to learn more of scientific logic and method, he will find the fullest satisfaction in an admirable work, "The Grammar of Science," by Karl Pearson (London, Second Edition, 1900). And the errors of the mind of man were long ago set forth with great power by Bacon as The Idols in his "Novum Organum."

To discover new facts is not the whole duty of the scientific man. It is also a part of his task to communicate them to his fellows. And so the student, aside altogether from the purely pedagogical necessity of showing to the teacher that he has done his work well, should learn to express forcibly and vividly his results to others. Such exposition has also the great advantage that it aids, as nothing else can do, to give clearness and definition to the student's own knowledge and ideas of the subject. He should cultivate all possible simplicity of style, and employ all available devices of illustration, using words, pictures, tables of figures, or graphs, according as to which is the more expressive for the particular point under discussion. In his elementary courses the student will have learned the usual form for recording the results of his experiments,—the definition of object, the description of method, the statement of results, and the discussion of conclusions. But in this course he should do more than this; he should learn to present his materials precisely as he would to a critical audience through a scientific magazine, taking as models some of the best published papers accessible to him. These as a rule give first, (*a*) a descriptive title; (*b*) an explanation of the status and importance of the problem; (*c*) an outline of the development of knowledge of the subject, with references to the principal literature, properly cited;* (*d*) a description of the methods and appliances used, with a discussion of their defects and of the probable sources of error; (*e*) the actual results observed, set forth in words, drawings, diagrams, graphs, or figures, as may be most expressive; (*f*) a summary of the results and their bearing. And these expositions should all be presented in a suitable and neatly kept book. The student will not be able to treat all of his topics so fully, but he should make this attempt with some of the most important. Needless perhaps to say, these expositions by the student should be carefully criticized by the teacher.

An essential part of scientific exposition is apt illustration, which in essence is simply an additional mode of expression.

* Rules for citation of literature, formulated by a Committee of Botanists and used by the principal Journals of this country, are in the Botanical Gazette, **20**, 1895, supplement to the March number.

The student will have learned in his earlier courses the characteristics of good scientific drawing,—that exact faithfulness to fact, that diagrammatic clearness, that complete accuracy in detail, which are so far from the impressionist effects sought by artists. In this course he will find that outline diagrammatic drawings will be of most use in illustration, especially of apparatus; and he should learn, through study of good models, to make them. Such drawings are of two kinds. *First*, there are the mechanical sections, used extensively in this book; they are easy to draw by aid of the simple instruments of mechanical drawing, and are valuable in showing the exact construction of apparatus. *Second*, there are external outline views, illustrated by figure 40 in this book; they are decidedly more pleasing than sections to the eye, but, involving perspective, are more difficult to draw, and they are also somewhat less illustrative of details of construction. These the student should learn to draw freehand, but he should also know how to make use of the semi-mechanical method of outlining in waterproof ink, and then bleaching, a photographic blue-print, details for the accomplishment of which will be found under Manipulation in Part III. He should learn also something of the modes of reproducing illustrations for publication, upon which there is a good article in Encyclopædia Britannica, **32**, 13, and of the methods of preparing drawings for such reproduction, upon which there is a very valuable article by BARNES in the Botanical Gazette, **43**, 1907, 59.

An invaluable kind of illustration is the graph, by which statistical data are recorded in curves or polygons. As illustrations graphs bear very much the same relations to tables of figures that pictures do to pages of words, that is, they not only express results clearly to the eye at a glance, but they also bring out facts and relations which would not be discovered by even a minute inspection of words and figures. Though valuable for representation of all quantitative relationships, such graphs are especially illuminating when two or more sets of data are to be compared. They 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 factor which alters steadily (as, *e.g.*, temperature), and the ordinates, or vertical lines, express the degree of the effect upon the plant. The joining of the tops of the ordinates, by carefully ruled lines, gives the polygon showing the relation existing between factor and effect, especially the rate of rise or fall of the effect under influence of the factor. In general the polygon is preferable, because more true to observation, than the curve, which is made by drawing a sweeping line through the tops of the ordinates; but the curve has its use for generalized or demonstration purposes. In the construction of the graphs there is no necessary relation between the values of the abscissæ and of the ordinates, but these may be so established as to give the most expressive result. As a rule the most striking form of graph is one in which the height does not exceed the spread of the base. The principles of their construction are well illustrated by the accompanying example (Fig. 1), made in the course of regular work by my own students. The average curve provides, on the principle earlier described, a standard of comparison and measure of probable error in the results of the individual. In illustrating a scientific paper by graphs, it is a good rule to give also the figures upon which it is based, all very compactly and neatly tabulated; for the reader may thus test for himself the accuracy of the graph, or he may wish to make other use of the figures.

The form of graph just described, which may be termed the rate-graph, is the one most used in Plant Physiology. There is, however, another form, called the frequency-graph, which is very useful wherever variability of any units is concerned. It is constructed by marking off the degrees, or classes, of the variable quantity along the abscissa, or horizontal line, and then reckoning one ordinate, or vertical, space for each individual falling under the respective degrees, or classes, after which the joining of the tops of these ordinates will give a curve, or polygon, of frequency. Such a curve is a refined substitute for a mean, for while the latter lumps together all the data regardless of any internal classification they may exhibit, and moreover is often made erroneous, or at least misleading, by the presence of very aberrent individuals, the former sorts the data out, so to speak, and arranges them around their center of frequency (mode), or, in some cases, their several modes. It is important in this connection to keep in mind an application of QUETELET'S Law, namely, that variables, where there is no disturbing cause, tend to group themselves symmetrically on both sides of their

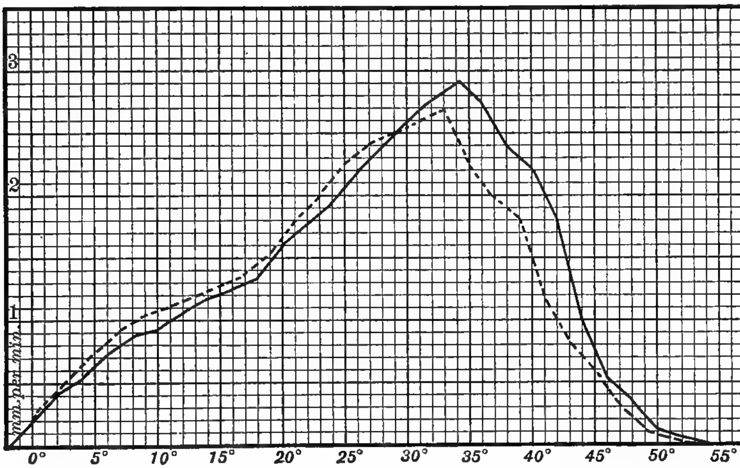


FIG. 1.—GRAPH (POLYGON) OF PROTOPLASMIC STREAMING IN A SPECIES OF NITELLA UNDER VARYING TEMPERATURES, RECORDED FOR ALTERNATE DEGREES.
The continuous line is an average of the results of nine students; the broken line is the result of one student.

mean, and also that they fall most abundantly nearest the mean. This principle has some important practical, as well as theoretical, uses in Plant Physiology, as will later appear.

More than once in this book I have emphasized the desirability of giving the educational study of Plant Physiology a more quantitative character than it has had in the past. An important phase thereof concerns the expression of physiological quantities, or constants, for the different processes. The physiologist cannot, like the chemist and the physicist, express his quantities in definite figures or formulæ, because plants are too variable both in themselves and in their relations to external conditions. Nevertheless, some form of expression of physiological quantities is certainly desirable if not essential, in order to give definition to knowledge of the respective subjects. Thus it is vastly better, for example, to know that green leaves in bright light in summer form about one gram of food substance (photosynthate) per square meter of leaf per hour, generalized and inaccurate for most plants though that statement is, than not to know anything at all definite about the amount. Accordingly in my own teaching, and in this book, I have determined for each process the mean of all the available quantities, have expressed this mean in the nearest round number (taking it in the direction of greatest probability or frequency), and have adopted this number as the constant of the process, giving warning of its nature, however, by calling it the *conventional constant.* In some cases it is desirable to know not only the mean but also the extremes of a process, in which case, whether expressing the exact quantities or their round numbers, I have linked the three together by hyphens with the mean in the middle in heavier type. Furthermore it is also desirable, both for convenience of comparison and also as an aid to the memory, to state all constants in as nearly as possible the same system of units, which should be capable of clear, brief expression. Accordingly I have adopted the standard of grams per square meter of area, or per kilogram of weight, or per liter of bulk, per hour, always stated in this order and expressed in brief as gm^2h, *gkh*, or *glh* respectively. The large units of meter, kilogram, and liter have the advantage of permitting most physiological quantities to be expressed in whole numbers, with a minimum of decimals. These conventional constants are not intended for investigation purposes, though some such use they may incidentally have; but they are provided for the education of the student, who should learn them by heart and have them ever ready for use. They cannot be misleading if their conventional character is kept in mind. A synoptical Table of those already worked out will be found in Part III.

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. Theorizing has this justification, that the current theories which 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, *first*, made personal acquaintance with the facts they are designed to explain, and, *second*, has tried to develop for himself some interpretation of those facts. Students receive with but a languid interest such theories as that of the micellar basis of membranes, or those which explain osmotic pressure, when these are formally presented to them; but they receive these theories with a very different interest when offered after they have tried themselves to devise an explanation of the facts they have observed in their studies upon those matters. 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.

Turning now to actual procedure in the conduct of the course, I think of but little to add. The individual work of the student in the laboratory, under the criticism and suggestion of the teacher, is its most important part, but this should be supplemented by the approved devices of lectures,* conferences, semi-

* For illustration of lectures, etc., wall diagrams 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 H. LAMERTIN, Brussels, and costing 50 francs). They may be imported through any dealer in foreign books.

On the projection of various physiological processes upon a screen, for demonnars, etc., for welding the scattered knowledge of the laboratory into a well-proportioned whole, and fixing it rightly and firmly in the memory. The course should involve a thorough drill in the essentials of physiological knowledge while fanning every spark of individual originality. Of course each student will have his own place and property, for which he is responsible. He should be given his problem, with full directions as to apparatus and manipulation, and should then be left to work out results for himself. He should be required to practice all new manipulation before applying it to his problem. He should be expected to complete particular problems, and to make full records, before bringing them for the teacher's inspection, since otherwise the student tends to rely more and more upon the teacher at each step, until finally the student is doing the mechanical and the teacher the mental work. But none of these pedagogical devices should be allowed to dominate the course or give it a spirit of formalism, but all should be subordinated to the liberal and co-operative spirit in which the work should be carried on.

A point of considerable importance in the conduct of the course concerns the relation of one student's work with another's. As to this there are two possible plans. In one, which is the older, the laboratory is provided with a piece of each of the approved and purchasable kinds of apparatus, and each student is assigned a distinct topic for somewhat thorough study; the topics are changed, or exchanged, two or three times in a term, and each student is expected to keep the others informed upon his subject, and likewise to learn from them. On the other plan, which I advocated strongly in the first edition of this book, the students all work together upon one topic, and each performs a series of experiments intended to cover the principal phases of the subject. This plan is only rendered possible by the use of simple and inexpensive appliances. Each method has advantages and drawbacks. The first, or indi-

stration to an audience, there is an important paper by PFEFFER in Jahrbücher für wissenschaftliche Botanik, 35, 1900, 711.

vidual, system gives better training, but less knowledge, since students, in fact, learn little from one another in this way. The collective system gives better knowledge, but poorer training, the more especially as the necessarily imperfect apparatus makes really accurate work impossible, and hence soon removes the desire for it. In my own experience during the past few years I have gradually approached a combination system, which seems to me to keep most of the advantages with only a modicum of the defects of each plan, and this I have had in mind in arranging the details of the second part of this book. In brief the students are always carrying on certain topics together, especially those of a markedly quantitative character, on which they compare results and to which the didactic part of the instruction is adjusted; at the same time they are each carrying on a special problem with much more thoroughness. It is now becoming possible to procure enough apparatus, and at moderate cost, to permit of profitable work upon this plan.

This discussion of the method of work in a college course in Plant Physiology brings up the question as to corresponding procedure in the elementary courses in Botany, involving the topics outlined on an earlier page (page 5). Where the classes are very large it seems at first sight quite impracticable to teach physiology at all, and certainly actual individual work is not possible. But after trial of different methods, I have had good success, even with over one hundred students in a class, by using a modification of the demonstration system, after the following plan.* With the entire class assembled, and only the bare materials for the experiment upon the table, I first do my best to make sure that the importance and general bearing of the problem is clearly before the students; in fact I try to make the experiment seem both a logical and a necessary step in their progress. Then I set up the experiment from the very beginning, explaining the reason for each step, the use of each piece of apparatus, and the action of each chemical involved.

* I have discussed the procedure more fully in School Science, 1, 1902, 463.

The students follow, asking, at regular times, such questions as they wish. When the experiment is complete and the results are ready, or when it is time for the application of some special test, the experiment is again brought before the class, the results are exhibited and discussed, and the tests are applied. The completed arrangement, showing the results of the experiment, is then placed in the laboratory, and each student is required to make a study of it, exactly as if he had performed it for himself. Then, using as a guide a mimeographed outline which suggests, but does not tell, the principal matters involved in the subject, the students are required to write, with proper illustrations, a clear account of the object, method, results, and general bearing of the experiment. The method is a fair, though not entirely satisfactory, substitute for actual work by the individual.

With so much that is attractive and important pressing for the attention of the student, it is necessary for the teacher to carefully seek out means whereby the best return in physiological training and knowledge may be derived from the time and energy the student can give to the subject, taking account, of course, of the many practical limitations imposed by cost of materials, difficulties of manipulation, arrangement of the college year, and the like. The ideal in this matter is the reconciling of all these factors to an harmonic optimum. The search for this optimum has been my pleasing task for some years past, and my experience is embodied in the outlines of Part II, upon the construction of which I would now offer some remarks. The work is all presented to the students as a series of problems, which are connected by comments and explanations designed both to show the relations and bearing of the subjects, and also to suggest the lines along which the scientific mind would naturally progress from one to another. I have aimed to include all physiological topics of consequence, and in general I have tried to treat them with an amount of emphasis directly proportional to their importance, subordinating by briefer treatment all lesser matters, no matter how simple or pleasing their experimental study may be. This plan I have modified in some cases where, as in Protoplasmic Streaming, in Photosynthesis, and in Transpiration, a certain topic or experiment seems to give exceptional opportunity for training in scientific ways of working, or for the acquisition of knowledge particularly important to the physiologist. To those problems or subjects, whether to be studied experimentally or through the literature, which seem to me of such fundamental importance that every student should fully know and describe them, I have given additional prominence by italic type, leaving matters of lesser importance without such emphasis. These latter topics, including the suggested experiments, will serve as good problems for individual students. I have sought always to make clear, by suitable introduction or comment, the theory of each experiment. In citing literature I have expressly avoided a duplication of anything in the admirable works of PFEFFER and of JOST, and have confined my references to such comprehensive or otherwise especially excellent papers as I think it particularly advantageous for students in this course to read. In many cases it will happen that, in his elementary course, the student will already have tried, or have aided in trying, some of the principal experiments, in which case he had better not repeat them, but, reviewing his earlier knowledge, he should concentrate upon matters new to him. To the form of the problems and their wording I have given much study, since through them the student's attention may be directed in the most profitable lines, his energy may be made most telling, and his attack on his topics may be made inductive, while through them, also, much both of suggestion and of stimulus may be conveyed. Since all physiological processes have their seat in Protoplasm, the course begins with a study of that substance; it then takes up the processes somewhat in the order of their dependence upon one another, Photosynthesis, most fundamental of all, coming first. But secondarily, attention is also given to the conditions of the college year, for which reason Growth and Irritability come last, in their proper place in the spring. The outlines embrace a full year's work under the most favorable conditions; where these are less fortunate, selection must be exercised.

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 investigators upon the same subject. The great accumulation of literature makes it necessary not only that the student shall be able to read absorptively and critically, but also that he shall acquire the power of obtaining some knowledge of a work by skimming its pages or by inspection of its tables of contents, figures, or summaries. In such a course as this, however, supposed to be taken by undergraduates, it is obviously impossible to carry the consultation of the original literature very far, especially if in a foreign tongue, but enough of this should be done to emphasize its value. Original papers can at least be brought into the laboratory frequently and looked over, even if not read. Happily, however, for such a course as this, the literature is admirably summarized in PFEFFER'S great handbook, "The Physiology of Plants." It is assumed throughout this course that the student shall constantly consult this indispensable book. It is, however, a difficult work to read in quantity, and by far the best reading book we possess on the subject is JOST'S "Lectures on Plant Physiology," a work which the student during his course should carefully read through.* He may consult to advantage also VINES' "Lectures" and SACHS' "Lectures," though they 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"; much of it is not botanical, but for the student's use it is none the worse for that. GOODALE'S "Physiological Botany," though much

* The English edition, unfortunately, is marred by many errors of translation, some so serious as to alter, or even reverse, the meaning of the original. Hence at all critical points the original should be consulted. See review in Nature, **77**, 1907, 97.

behind the present state of knowledge, continues to be a book which students consult by preference as a work which tells things as one wishes to know them. Very valuable for its summaries of experimental methods and statistical results, for both animals and plants, is DAVENPORT'S "Experimental Morphology." SORAUER'S "Popular Treatise" has distinctive value for the economic aspects of the subject. SCHIMPER'S "Plant Geography" is indispensable for the ecological phases of physiology. A recent and readable summary of the subject is GREEN'S "Introduction to Vegetable Physiology," and of much the same scope is PEIRCE'S "Text-book of Plant Physiology," while for a brief synopsis there is nothing so good as NOLL'S "Physiology" in the Bonn Text-book. CLEMENTS' "Plant Physiology and Ecology" (which includes the material of his earlier "Research Methods in Ecology") describes some new appliances and methods especially applicable to outdoor work. Again, it is very profitable, and at times indispensable, to consult other works upon the experimental phases of the subject, and for this there are two admirable books, DETMER'S "Practical Plant Physiology" (together with his more recent "Kleines Praktikum," still untranslated) and DARWIN and ACTON'S "Practical Physiology"; while MACDOUGAL'S "Practical Text-book of Plant Physiology" is an excellent work, containing some matter not elsewhere accessible.

The above-mentioned works are such as would be used by students in a college course in Plant Physiology. In addition there are others which, dealing with the simpler and qualitative phases of the subject, and recommending adapted or makeshift appliances, interest especially the teachers in the schools. Among the most recent and excellent of these are ATKINSON'S "First Studies of Plant Life" and MACDOUGAL'S "Elementary Plant Physiology" (to which may be added his non-illustrated "Nature and Work of Plants"). More recently has appeared an excellent German book, LINSBAUER'S "Vorschule der Pflanzenphysiologie." Most prominent of all books of this type, however, and apparently well-nigh exhaustive of its particular field of simplified Plant Physiology, is OSTERHOUT'S "Experiments with Plants." These books, with all of the above-mentioned, are contained in the following list.

CLEMENTS, F. E. Plant Physiology and Ecology. New York, Henry Holt & Co., 1907. $2.00.

DARWIN and ACTON. Practical Physiology of Plants. Second ed. Cambridge, 1895. 4s. 6d.

DAVENPORT, C. B. Experimental Morphology. Parts I and II. New York, The Macmillan Co., 1897, 1899. I, $2.60. II, $2.00. A new issue in one volume, 1908. $3.50.

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

—— Das Kleine pflanzenphysiologische Praktikum. Jena, Gustav Fischer, 1903. M5.50.

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

GREEN, J. REYNOLDS. An Introduction to Vegetable Physiology. Philadelphia, Blakiston's. Second ed., 1907. $3.00.

JOST, L. Lectures on Plant Physiology. Translated by R. J. H. Gibson. Oxford, Clarendon Press, 1907. $7.75.

MACDOUGAL, D. T. Practical Text-book of Plant Physiology. New York, Longmans, Green, & Co., 1901. $3.00.

NOLL, F. In Strasburger, Noll, Schenck, and Karsten, A Text-book of Botany. Translated by W. H. Lang. New York, The Macmillan Co., 1908. $5.00.

PEIRCE, G. J. A Text-book of Plant Physiology. New York, Henry Holt & Co., 1903. $2.00.

PFEFFER, W. The Physiology of Plants. Translated by A. J. Ewart. Oxford, Clarendon Press. Volumes I–III. 1900–1906. $17.75.

SACHS, J. Lectures on the Physiology of Plants. Translated bv H. M. Ward. Oxford, Clarendon Press, 1887. (Out of print.)

SCHIMPER, A. F. W. Plant Geography on a Physiological Basis. Translated by W. R. Fisher. Oxford, Clarendon Press, 1903. $12.00.

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

VERWORN, M. General Physiology. Translated by F. S. Lee. New York, The Macmillan Co., 1899. $4.00.

VINES, S. H. Lectures on the Physiology of Plants. Cambridge, University Press, 1886. 21s.

To these may be added, as a supplement, a list of the more important works devoted to the simpler (elementary, or Nature Study) phases of the subject.

ATKINSON, G. F. First Studies of Plant Life. Boston, Ginn & Co., 1901. 60 cents; 85 cents.

LINSBAUER, L. and K. Vorschule der Pflanzenphysiologie. Wien, Carl Konegen, 1906. M4.50.

MACDOUGAL, D. T. Elementary Plant Physiology. New York, Longmans, Green, & Co., 1902. $1.20.
—— The Nature and Work of Plants. New York, The Macmillan Co., 1900. 80 cents.
OSTERHOUT, W. J. V. Experiments with Plants. New York, The Macmillan Co., 1905. $1.25.

3. GREENHOUSE AND LABORATORY FOR PLANT PHYSIOLOGY.

It goes without saying that an experiment greenhouse and laboratory are indispensable for extended and efficient work in Plant Physiology. Something can be done, it is true, with Wardian cases, enclosed bow windows, or other simple arrangements, but these should be used only as temporary stages in progress toward a suitable equipment. Buildings and tools alone will not ensure the best results, but neither will goodness of spirit in teacher and students. Results which are worth while come, for the most part, from a combination of material facilities with human devotion.

I shall now describe a greenhouse and laboratory which seem to me adequate for good work by a dozen students in such a course as is described in this book, or, better, by six or eight students, with facilities for some research by the teacher and one or two graduate students. It happens that I can base my recommendations, not upon theoretical likelihood alone, but upon actual practical experience; for I have had the good fortune to be able to build, with only very reasonable financial restrictions, just such a greenhouse and laboratory as I wished. The account which follows is a description of the carefully planned and thoroughly built physiological equipment of Smith College, with comments upon such features as my five years' use of it have shown could be improved.

The greenhouse and laboratory adjoin one another and form part of a range of several houses devoted to educational work. This arrangement economically provides the plant material, the heat, and the skilled care for their proper maintenance.

It is no part of my present subject to describe these houses, but if the teacher cares for such information, he may find the complete Smith College equipment (the Lyman Plant-house, a memorial gift) described in Science, **15**, 1902, 933.

The experiment greenhouse has the form and arrangement shown by figures 2, 3, 4, and by the accompanying photographs, Plates I and II. It is 32 by 19 feet,* inside measure, with solidly founded brick walls rising 3 feet 6 inches from the floor, above which are glass sides to 6 feet, while the glass roof rises in the center to 12 feet 6 inches. Ample ventilators are provided on walls and roof, and care is taken that the ventilator rods do not project into the room beyond the walls. The floor is everywhere cemented, and no special arrangement for drainage is needed, since evaporation and occasional mopping accomplish this result. The heating (hot-water) pipes, fully controllable by valves, are ten, of 2½-inch diameter, arranged along the walls as shown in figure 4, but include four more in number than are needed. Upon the pipes are shallow trays of galvanized iron, kept filled with water, which helps to moisten the air; but on dry, warm days these must be supplemented by copious sprinkling (by hose) of the cement floor. In this feature, however, the house could be improved, and I have no doubt it would be better if the pipes were sunk below the floor in brick or cement pits (covered by iron gratings), so arranged that water could constantly evaporate from them; upon occasion, additional moisture could be quickly provided by spraying the pipes themselves.

So much for the house itself, which, as a whole, is most satisfactory in use. We consider next its furnishings.

First are the tables, which must be very solidly built in order not to move when touched or pushed. They are in number as shown by figure 2, are 3 feet high (the best for work while

*That I use feet and inches for laboratories and furniture, while keeping to the metric system for all scientific measurements, is due neither to oversight nor lack of desire for universal extension of the metric system, but to the feeling that it is the most convenient and sensible way under present conditions.

View in an Experiment House for Plant Physiology. Looking towards the Laboratory.

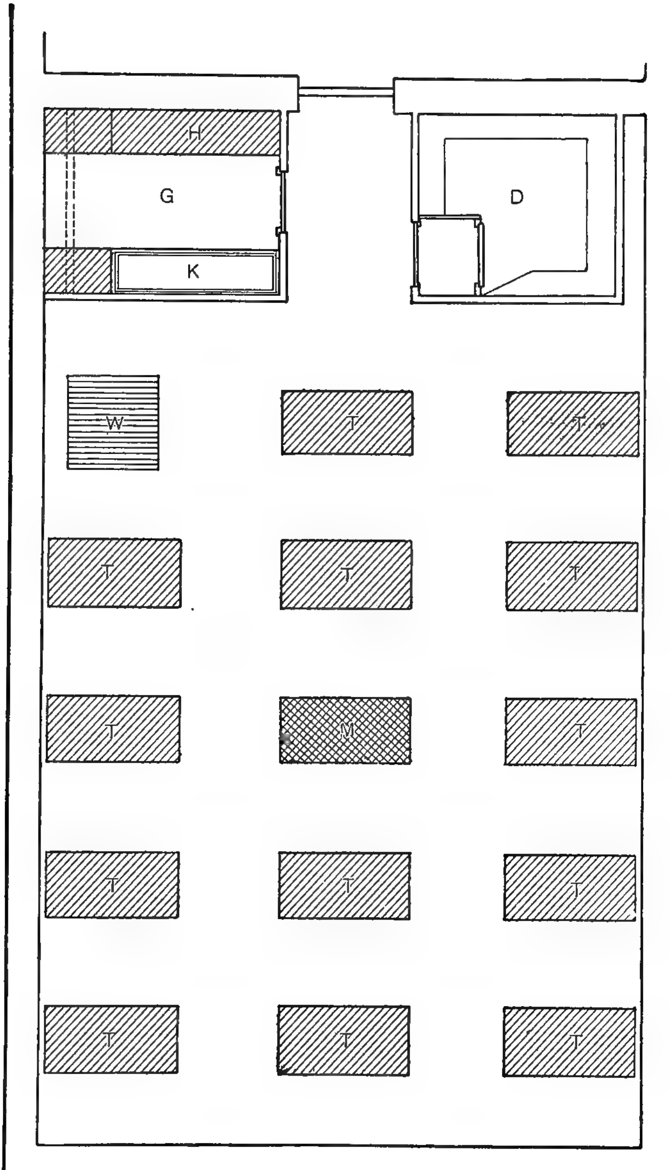


FIG. 2.—GROUND PLAN OF EXPERIMENT GREENHOUSE; $\times \frac{1}{72}$.

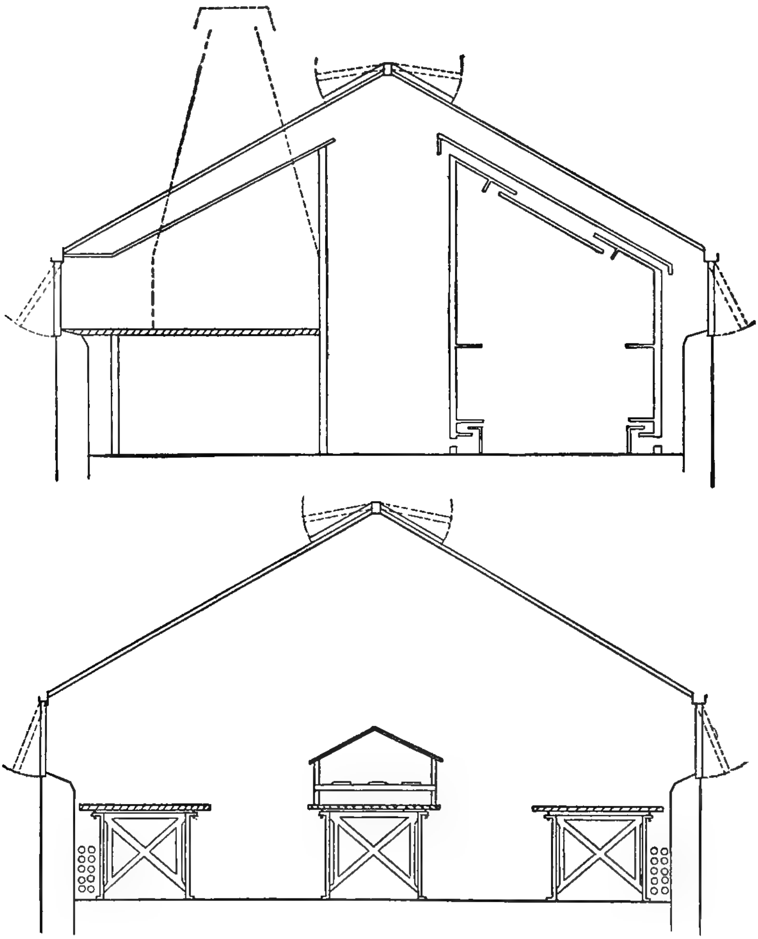
D, physiological dark room; *G*, gas (and water) room; *H*, heating table under hood; *K*, sink; *M*, meteorological instrument table; *T*, tables for experiment; *W*, Wardian case (or meteorostat).

standing), have tops of solid slate, 2×4 feet by $1\frac{1}{2}$ inches thick, resting on 4 adjustable nuts, and have a very solid cast-iron stand of the form shown by figure 4 (the end view being somewhat similar, though narrower, and with a vertical central piece).* Of the very greatest importance is a physiological dark room, in which plants may be kept in complete darkness, but under good ventilation. Its plan is shown by figures 2 and 3. It is wholly inside the greenhouse, whose average temperature it takes and keeps very constantly. It is built of one thickness of brick ($3\frac{1}{2}$ inches), and has an entrance-way, made of matched boards, so arranged that by opening one door at a time it is possible to enter and leave the room without admitting any light. The doors fit tightly into angled cases, and are further made light-tight by rubber weather strips. The alternate bricks, one row above the floor all around (except at the entrance), are omitted, and over the openings is built the arrangement of boards wholly painted black, shown in section by figure 3; this freely admits air, but no light. The same end is accomplished in the roof by the triple arrangement shown in section in figure 3. This roof is black throughout except on the top, where it is painted white to reflect as much heat and light as possible. Shelves at convenient heights are added (the lower ventilation box serving as one set), and an incandescent electric light permits the room to be lighted when desired. In use it has proven wholly satisfactory.

Another very important furnishing is a large sink. In the greenhouse I am describing this is placed against the laboratory wall in the position of the hood shown by figure 3, but I can suggest a much better place for it, as noted below. I have discovered one, and only one, marked defect in this greenhouse and laboratory equipment, *viz.*, it has no suitable, but only a small makeshift, place where heating can be done in such

*These tables were designed, and other good features of the houses were suggested, by Mr. W. A. BURNHAM (of the firm of LORD and BURNHAM, New York, builders of the houses), who took a deep and generous interest in the development of these houses as an educational equipment.

a way that steam, gas-fumes, vapor of chemicals, etc., often developed in laboratory work in physiology, can escape. Ac-



FIGS. 3 (UPPER) AND 4 (LOWER).—CROSS-SECTIONS THROUGH EXPERIMENT GREENHOUSE, AT DARK AND GAS ROOMS AND AT CENTRAL TABLES; $\times \frac{1}{72}$.

The various features are explained by Fig. 2 and the text.

cordingly, I have designed, and later hope to add, the special room shown in figures 2 and 3. It is to be built inside the greenhouse, answering in general form and size to the dark room, with walls of a single thickness of brick up to 3 feet 6 inches; but above this it will be of glass to the roof, which is to be made of thin, matched boards, painted white above. Inside, against the laboratory wall, will be a slate table holding several Bunsen burners with their supports, and above it a metal hood leading to a protected outlet, as indicated in figure 3. This should ensure the removal of all gases and vapors from the building. Along the wall towards the greenhouse will be the long porcelain-lined sink with the several taps needed for the different appliances described later under Apparatus. The short table between the sink and the ventilator is intended for the gas generators with pneumatic trough, while shelves above the sink are to contain the chemicals in actual use. Ventilation will be provided by the side ventilator, made a part of the room as shown by the figure. Of course the door into the laboratory will close tightly. Being practically wholly inside the greenhouse, it will need no special heating.

In any working experiment greenhouse, it is desirable or essential to have a set of recording meteorological instruments, the nature and use of which will later be considered. These are placed on the central table under a simple but efficient cover made of wood painted white, of the form shown in section by figure 4, and in view by Plate I.

The necessary shading of the house against too great light and heat is provided in part by a coat of whiting placed on the outside of the glass in February or March; it weathers off before the next winter. But an adjustable shading over the south side is given by cotton shades, which rest upon wires close to the roof, and are drawn up by cords running over pulleys. The system does not, of course, give intermediate gradations of light, as could be provided by some system of swinging shades, but it has proven efficient for all ordinary purposes.

The final furniture of the experiment greenhouse consists of a case in which, for special purposes, light temperature and moisture, especially the two latter, may be kept stable or varied at will, a modification of the Wardian case which may be termed a Meteorostat. My own is shown in position by the photograph II. It is wholly of glass and metal, 2½ feet square, 4½ feet high to the peak of the sloping roof, and rests on an iron support 2½ feet high. It is not air-tight, but would be better if it were. Originally it was warmed by water in a copper box, 4 inches deep, forming its bottom, the water being heated from beneath by a gas-flame controlled by a thermo-regulator in the case. To prevent the escape of gas-fumes into the house, the flame was enclosed by an air-tight hood with inlet and outlet tubes, 6 inches wide by 1 inch thick, leading out-of-doors. More recently I have heated it by electricity from an incandescent circuit, the current passing through a heating coil in a smaller copper box of water in the bottom of the case, though this, owing to defects in the regulator,* has been only partially satisfactory. Cooling is effected by a coil of tin pipe in the top of the case, through which cold water from the street pipes can be circulated; this suffices to prevent undue heating when the temperature of the house rises too high on bright days, though it is insufficient to keep the case permanently much below the average temperature of the house. To promote a more rapid and even warming or cooling, small electric fans driven by a battery below the case are installed beside the warming box and the cooling coil, and their occasional use equalizes the temperature throughout the case. Humidity is readily raised by placing a wet sponge in front of one of the fans, and lessened by exposing shallow pans of calcium chloride, though a tube through which air could be driven over the calcium chloride by one of the fans would be better. Light is tempered by a white curtain, and cut off altogether by a hood, completely enclosing the case, made from double black sateen. A recording thermograph and a hygrograph complete the arrangement.

This meteorostat, though better than any other arrangement I have seen, and ample for all student work, is yet only moderately efficient; and I have designed a much better one, adapted

* Simple, but apparently efficient, electric thermo-regulators are described by MAST in Science, **26**, 1907, 554, and by CANNON in the Plant World, **10**, 1907, 262.

to investigation, which I hope some day to install. It is to be composed of tightly jointed plate glass, projecting in part from a southern exposure of the greenhouse, where it is to be covered by an outer case of plate glass, the intermediate space, one foot deep all around, serving for circulation of warm air from the house, and also to hold shades for regulating the light. The plants and instruments will stand on adjustable glass shelves, where they will be lighted from the east, south, and west sides, as well as from above. It is to be 6 feet high by 4 feet square, and therefore large enough for a person to enter (through a large port hole in the inclined bottom) for the arrangement of the apparatus. The temperature, moisture, etc., are to be regulated by drawing the air of the case through accessory metal chambers by electric fans, and these chambers are to be, respectively, (*a*) heated by electric coils, (*b*) cooled by metal boxes containing ice and salt, (*c*) moistened by wet sponges, (*d*) dried by calcium-chloride or sulphuric-acid troughs. I have no doubt the arms of the registering thermograph and hygrograph can be made, as they rise and fall, to open and close circuits which will automatically start and stop the appropriate fans, thus making the whole arrangement self-regulating. It is only by means of such a chamber, in which the conditions can be controlled and varied one at a time, supplemented by autographic instruments, that we can separate and determine the effects of the individual external factors upon the plant.*

Such is an efficient experiment greenhouse, thoroughly built for long service. At present prices it will cost close to $2000, supposing the heat to be available from a neighboring house. Here, as elsewhere, the best is, in the end, the most economical. It can be built much more cheaply by using a wooden instead of an iron frame, simpler tables, and other economies. Indeed, greenhouses can be built of almost any degree of cheapness, and I have been told of one, said to be really efficient, which reaches the limit of simplicity; it is sunken in the ground

* For studies requiring simply an even temperature and humidity, with darkness, an underground chamber would undoubtedly be best.

almost to the roof, which is composed simply of commercial sash arranged to be raised for ventilation. All builders of greenhouses describe simple heating arrangements for small houses. Sometimes the greenhouse is built on a roof or in a favorable angle or court of the laboratory building, but such houses have to contend with certain drawbacks,—the labor of transporting soil, pots, etc., up and down, the presence of gases rising with warm air in the building, the difficulty of securing proper arrangements for abundant wetness, and, usually, a difficulty about heating. Nearest the ideal is a house contiguous to the laboratory, but in attachment to a range of educational houses, as in the case of the one I am describing. There are descriptions of other experiment houses mentioned by DETMER in his "Practical Plant Physiology," page 8, and there is a good description of new physiological experiment houses at Bonn (Germany) by Lloyd in the Journal of Applied Microscopy, **5**, 1902, p. 1829. There is also a valuable article upon greenhouse construction in Bailey's "Cyclopedia of American Horticulture."

The management of an experiment greenhouse, to keep the plants therein under conditions for normal and healthy growth, requires care, and some one person should be responsible for this. The greatest danger comes from excessive dryness, especially on bright, warm days, when the house may approach the conditions of a desert. Then such simple devices as pans of water on the pipes are ineffective, and the best remedy is to copiously wet down the cement floor with hose at eight or nine o'clock, and again at noon, unless, indeed, the pipes are sunk below the floor, as earlier suggested. It is also a good plan to have as many plants as possible in the experiment house, and it is sometimes advantageous to surround the plant under experiment by a wall of other plants, which devices help to keep more natural conditions of moisture. It is also well to have in this house a form of simple hygrometer adjusted to agree with a similar one in an adjoining house filled with thriving plants; then, if the two are kept approximately alike, the moisture conditions will be about correct. Again, all fumes and vapors, especially gas-fumes and escaping gas, must be kept from the house, since, although some plants do not mind them, others are thus very much, or even fatally, injured.* To prevent both the drying out of the house, and also any possible entrance of gas, the doors to the laboratory, and, especially, to the gas room, should be kept always closed. No doubt in the former case a tight-fitting door opening either way with a push would be best. Equally important with the treatment of the house is the care of the plants under experiment, which the student must himself learn to attend to. Each plant should receive individual care and attention, and should be kept clean, watered, and generally well groomed. The golden rule of watering should be followed, namely, to give a copious watering at intervals, allowing the soil to partially dry out between times, rather than to give a little water often, which prevents free aeration and respiration of the roots. It is possible to tell whether or not plants are in need of water by the gardeners' test of tapping the pot, a "ring" meaning a need for more water, and a dull sound indicating that it still has enough. Further, the plants should never in winter be given water taken direct from the tap, for this chills them; but in the house there should always be a large dish or tub of water kept standing long enough to take the house temperature, and from this the water should be dipped and applied by a small watering-pot. It is sometimes a good plan, in order to prevent too great drying out of a pot, to place the latter in a larger pot with moist sphagnum moss between. Finally, as to the appearance of the house, it should not be allowed to show rust or neglect, but should be thoroughly scrubbed, the floor often, and the walls and roof once a year, and it should be kept painted white inside and out. This is not simply for its preservation, but also for the physical comfort and the moral support of the workers therein.

For a thorough college course in Plant Physiology, some form of greenhouse is essential, but a good deal can be done in

* There is an experimental study of this subject by RICHTER in Berichte der deutschen botanischen Gesellschaft, 21, 1903, 180.

elementary courses by use of a Wardian case, which should be placed before a large (preferably a bow) window. Plants will thrive in such a case, some kinds even better than in larger spaces. The case should be as nearly air-tight as possible in order to shield the plants from the worst of the gases of the room, and should preferably be ventilated at intervals by fresh air from the window. If the room is kept constantly warm all around

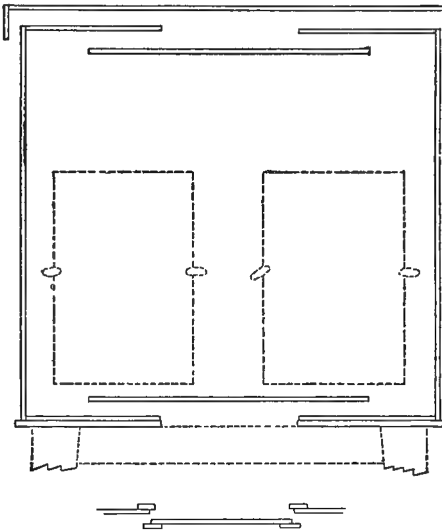


FIG. 5.—VENTILATED DARK BOX FOR PHYSIOLOGICAL USE, IN LONGITUDINAL SECTION; $\times \frac{1}{24}$.

The doors and table are dotted, and the lower figure shows the construction of the doors, which are held in place by buttons. The case is half as deep as it is broad.

the case, no artificial heating will be required, but if the temperature falls very low at night, the case should be heated either by a gas-jet beneath a galvanized-iron box forming the bottom of the case, or, much better, by an incandescent electric heating coil controlled by one of the thermo-regulators earlier mentioned (page 37, note). I have given somewhat fuller details concerning Wardian cases and their construction in my "Teaching Botanist," page 82. In such a case, as I know by trial, many of the simpler physiological experiments with living plants can be carried on perfectly, to such a degree that even some college work is possible with them when no better arrangements are available.

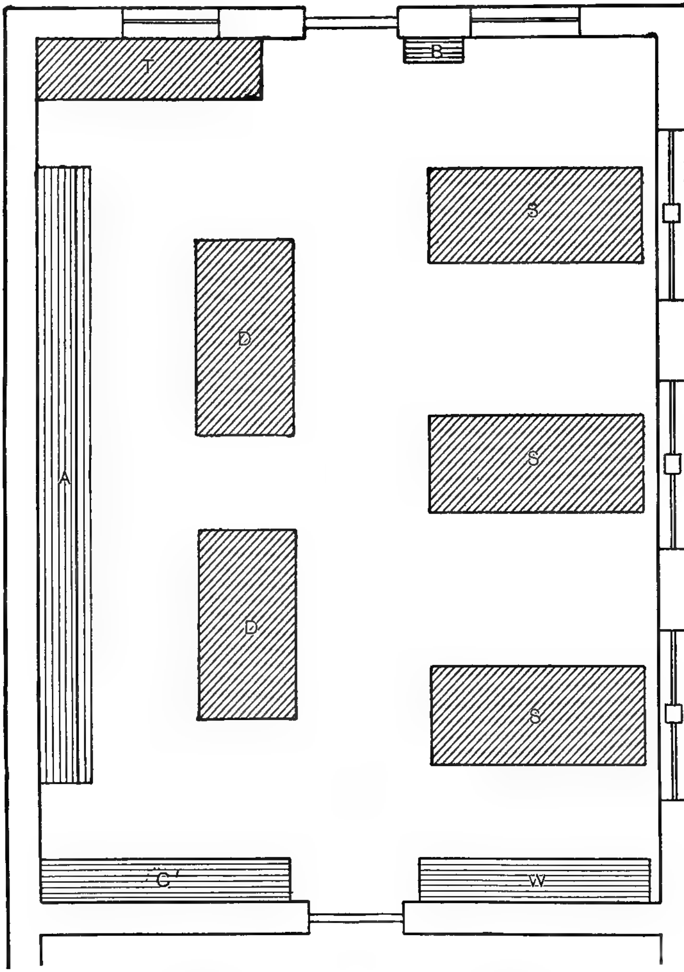


FIG. 6.—GROUND PLAN OF PHYSIOLOGICAL LABORATORY; $\times \frac{1}{72}$.
A, apparatus cases, with drawers beneath; *B*, bookcase, *C*, chemicals' case, *D*, drawing and assembling tables; *S*, students' work-tables; *T*, tool-table; *W*, case for balances.

It may happen at times that greenhouse space is available, but no place for a proper dark room. In this case a fair sub-

View in a Laboratory for Plant Physiology. Looking towards the Experiment House.

stitute is offered by a large, ventilated dark box, black inside, but white outside. The accompanying figure 5 shows in detail such a box (large enough to admit a person who could make sure of its tightness to light), which I used with entire success before I had a dark room at command.

Passing next to the laboratory, which should directly adjoin the greenhouse, we find its construction much simpler. The

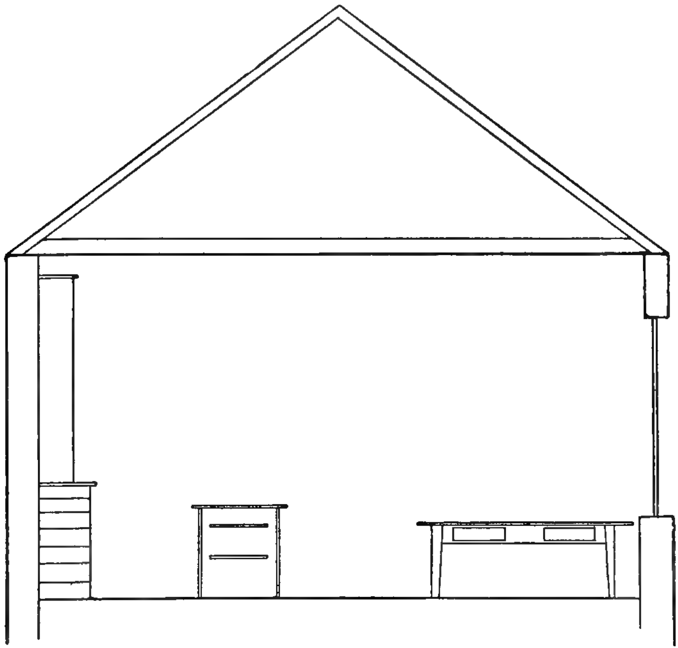


FIG. 7.—CROSS-SECTION THROUGH PHYSIOLOGICAL LABORATORY; $\times\frac{1}{72}$.
The various features are explained by Fig. 6 and the text.

one I have had built, and which has proven perfectly efficient, is shown by figures 6 and 7 and by Plates III and IV. It is built of brick and heated by pipes similar to those of the greenhouse, though fewer in number and arranged only under the windows. The students' work-tables are of the very simplest pattern. Of the greatest convenience are the central tall tables used for drawing, for the assembling of apparatus, etc. They are 3 feet high, for convenience in working standing, and have capacious cupboards beneath for storage of the larger glassware, etc. The apparatus cases, taking the larger and most valuable pieces, have glass doors, while beneath them are many drawers (each with a check block to prevent disaster if pulled too far out) for the innumerable smaller articles needed in the laboratory. There are special cases for chemicals and for books, and another, fixed against a brick wall without contact with the floor, for balances. An important table is the tool-table; it is 3 feet high and has abundant drawers beneath. For the storage of larger apparatus, I have utilized the loft of the laboratory, which is reached by a balanced dropping stairway built into the ceiling of the laboratory itself. Such a laboratory, with its furniture, would cost at present prices some $1500 or over. As thus planned the laboratory may seem to make too little provision for work in physiological chemistry. But I think that, on the one hand, it does afford, in conjunction with the gas and water room, facilities for all the chemistry which naturally belongs in connection with a college course in physiology and in simpler research, while, on the other hand, advanced work in physiological chemistry hardly needs to be done in connection with a greenhouse, but can best be carried on in a properly equipped chemical laboratory. In any case such work must at least be done in a separate room, which could be added as an ell of the laboratory.

4. APPARATUS AND MATERIALS FOR PLANT PHYSIOLOGY.

In a general way, according to the idea dominant in its construction, apparatus for Plant Physiology may be classified into four kinds, though there are many combinations and intermediate gradations. These kinds are:

(1) *Precision Apparatus*, made for research, in which the best possible is the only profitable kind. As a rule it cannot be purchased, but must be made to order at large cost by the most expert workmen.

View in a Laboratory for Plant Physiology. Looking from the Experiment House.

(2) *Normal* (or *Standard*) *Apparatus*, made by competent workmen expressly for its particular work, applicable thereto with convenience and celerity, yielding quantitative results of negligible error, and purchasable at any time from the stock of a supply company. This is the kind best for individual work in a course in Plant Physiology, and for some of the purposes of an elementary course.

(3) *Adapted Apparatus*, made up from approximately suitable articles or appliances, especially those sold for Physics and Chemistry, these being altered to fit their special work, either in the laboratory or by aid of local carpenters, etc. When carefully made they may be preserved and used year after year. Yielding results qualitatively correct, though quantitatively crude, they are useful for demonstration, and hence serve well for an elementary course in the science, as well as for some purposes in the higher course. They are also justified by their lower cost where one cannot afford a better sort, or where the latter is not yet obtainable.

(4) *Make-shift Apparatus*, brought together from various common articles temporarily pressed into the service, yielding results not at all quantitative and only crudely qualitative, and justifiable only in lower grade, or nature study, courses, where the doing of things has more meaning than the results.

It is my belief, representing a conversion from a different view held earlier, that in a college course in Plant Physiology emphasis should be laid upon the acquisition and use of normal apparatus, though by no means to the exclusion of either precision or adapted types. It is rather the spirit than the letter of the normal apparatus that should be kept prominent, and for reasons which I have already explained earlier in this book (page 11). I would simply repeat here, in this different connection, that the superior merit of the normal apparatus consists in three things: (*a*) in its ever-readiness for use, conserving time and energy for concentration upon the phenomena to be studied; (*b*) in the feeling of security and definiteness in progress accompanying the respect and confidence inspired by its accurate results; and (*c*) in its permission of the constant use of quantitative methods, which are the only prolific ones in the advanced phases of any science.

A very obvious reason for the comparatively small use of normal apparatus in the past has been the small amount of it that was obtainable, and this deficiency has proved a most serious hindrance to the progress of Plant Physiology. It is for this reason, and no other, that I have undertaken in recent years to develop normal apparatus for the use of my own students; and, in order to secure its skilled construction, and at the same time to make it generally accessible to all who may care for it, I have handed over its manufacture to the Bausch and Lomb Optical Company of Rochester, N. Y., in whose hands the business arrangements wholly are. So far I have prepared some sixteen pieces, which are described in this book, and of which an illustrated catalogue may be obtained from the makers, while other pieces are in advanced preparation. It is my hope to develop efficient pieces for each of the principal physiological processes. The construction of such apparatus has also to some extent been taken up by other students, as will be noted in a paragraph at the end of this chapter.

Another obvious difficulty in the acquisition of much normal apparatus is its expense. Undoubtedly it is expensive, as the equipment for all good scientific education necessarily is. But the expense is really less than at first sight appears. As readily develops from a comparison, the cost of fully equipping a laboratory of Plant Physiology is no greater than, indeed is not so great as that of equipping a good anatomical laboratory, with its microscopes, microtomes, and other accessories, and, moreover, everything is permanent and can be used many years. Furthermore all the pieces need not be acquired at once, but they can be obtained gradually, a few each year.

Another matter of importance in connection with apparatus concerns its terminology, upon which there is desirable just so much uniformity and system as will contribute to economy and efficiency. There already exists a certain good tendency in this direction, which if recognized and followed will serve the purpose well. Thus the names of appliances which simply exhibit the occurrence of a process are often distinguished by the termination *scope*, *e.g.*, hygroscope, diaphanoscope, to which I have added osmoscope, respiroscope. Also names of appliances which permit a process to be measured are indicated by the termination *meter*, *e.g.*, thermometer, osmometer, to which I have added respirometer, photosynthometer. Also names of appliances which record or register the progress of a process, that is, which are autographic, are designated by the termination *graph*, *e.g.*, thermograph, hygrograph, barograph, to which I have added transpirograph and auxograph. Again, from another point of view, names of appliances which keep a certain condition fixed or constant often have the termination *stat*, *e.g.*, thermostat, clinostat, to which I have added meteorostat and hydrostat. It is obviously desirable that this simple and expressive terminology be followed in naming new apparatus in the future.

The foregoing comments apply for the most part to apparatus used by the student for his individual experimenting. This apparatus will be described in the proper places in the course in Part II. But in addition there are certain articles which are much in use, and by all the students, and which belong really to the furnishing of the laboratory as a whole. Of these the more important are the following:

GAS-TABLE. This essential part of the furnishings should stand in a room where its fumes cannot reach laboratory and greenhouse (page 36), and should be covered by a ventilating hood rising from a glass case provided with sliding doors, as usual in chemical laboratories. It should be six feet long and two wide, with a top of stone, slate, or other fireproof material, raised three feet from the floor, while beneath should be drawers for the accessories, including glass tubing. It should be provided with Bunsen burners for (*a*) a still affixed to the wall, (*b*) a steam sterilizer always in position, (*c*) a water-bath, (*d*) a blast worked by a foot-bellows, (*e*) a tripod with asbestos support for beakers, etc., (*f*) a fish-tail burner for bending glass tubing, and (*g*) an ordinary jet for the working of the same. A soldering apparatus, a very small blowpipe for breaking glass tubing, and an arrangement for quickly securing a supply of hot water are also desirable accessories.

SINK. An indispensable part of the furnishings. It should stand preferably in a room apart from laboratory and greenhouse, though this is not essential as in the case of the gas-table. It should be porcelain-lined, eighteen inches wide, five feet long, and two and a half feet from the floor. It should be provided with several taps for (*a*) ordinary water-supply, to which a hose can be attached for wetting down the greenhouse (page 39), (*b*) a small nozzle to which small rubber tubing can be attached, (*c*) a Chapman exhaust, permanently attached, (*d*) a small water-motor with emery-wheel, very useful for sharpening instruments, grinding glass, etc., (*e*) a water-blast for aerating cultures and the like. Beneath the sink should hang, ready for use, two or three granite iron pans for washing, and a pneumatic trough; and over it should stand, in proper racks, three or four different sizes of cylindrical graduates, and a series of different sizes of funnels. Here also should be placed glass shelves for the chemicals most in use.

TOOL-TABLE. This may well stand in a corner of the laboratory before a window. It is used for simple carpentry and metal-working, and, in general, for the construction or adaptation of apparatus. It should have a solid wooden top, two inches thick, two feet wide, six feet long, and three feet from the floor, and should have ample drawers below for the reception of tools and the proper supplies. On it should be a vise, and over it should hang spools of copper wire.

STEAM STERILIZER, for sterilizing pots, saucers, moss, and other germination appliances, and for other similar uses. A very good form is the Arnold (sold at moderate cost by supply companies), of which the largest size is the most useful. It should stand always ready for use on the gas-table. For articles or materials often needed in sterilized condition, it is time-saving to provide a large glass jar with ground cover, into which they are introduced sterilized and hot, and from which they may be withdrawn as needed.

STILL, for providing the essential distilled water. One of the several automatic forms is best, and it should be permanently attached to the wall over the gas-table, with suitable water connections. Of course where steam-heat is used, it is more economical to condense the distilled water from the steam-pipes by carrying the steam through a pipe cooled by water from a tap. For some special purposes, stills which yield traces of metals will not answer, and, to provide for this, some one of the glass forms now offered by supply companies should be on hand ready for use.

AIR-PUMP. For practically all purposes the most convenient is a Chapman exhaust, which, attached to a water-tap yielding a good pressure, will give rapidly within one per cent of a vacuum. It should be connected with a gauge, preferably a mercury manometer affixed to the wall, which has the advantage that it renders the exhaust directly visible.

ASPIRATOR AND AERATOR, necessary for drawing air in some phases of gas analysis. For this the Chapman air-pump can be used in some cases, but where gentler and steadier action is necessary, one of the standard aspirators, sold by all supply companies, should be used. *Or*, a very good form may be adapted from two large bottles on the principle illustrated by the accompanying figure (Fig. 8). If the filled upper bottle is allowed to siphon through the rubber tube into the empty lower (the flow being regulated by the interposed glass stop-cock, or else by a screw pinch-cock), the upper open tube gives an aspirator or exhaust current, and the lower a blast or aerator current. The bottles may very readily be transposed,—by a pulley arrangement if desired.

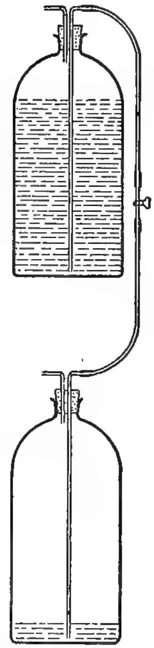


Fig. 8.—Aspirator and aerator; $\times\frac{1}{15}$. Particulars in text.

Blast. Needed for developing great heat in glass-working, for aerating water-cultures, etc. For the former a foot-bellows blast, sold by all supply companies, is best, and for the latter, various forms of glass or of metal, working by water-pressure, are offered by supply companies, the Boltwood form being considered very efficient. For most purposes an excellent blast is given by the aspirator just described. A simple arrangement for aerating solutions is described by S. O. Mast in the American Naturalist, **38**, 1904, 655.

Balances. Of these it is best to have three or four forms for the markedly different uses, and they should, the best of them at least, be kept in a case, permitting their use in position, attached to a solid wall of the laboratory without connection with the jarring floor. My own case is shown in the photograph of the laboratory on Plate III. For weighing chemicals a Troemner Balance (costing about $7.00) is very good, and should be kept in the case for chemicals. A spring balance, though too inaccurate for any scientific use, has many conveniences for rapid weighing, and a good form is the Mail and Express Balance of the Pelouze Scale Company of Chicago (cost $5.00). For a large balance taking heavy weights, and having long pan supports, the Gerhardt 3423 (costing 115 marks) is excellent. The Springer Torsion Balances, having the beams under the pans, are also good, though less delicate. For a sensitive large balance, which at the same time will take plants, etc., of any height, my own transpiration balance among my normal apparatus (page 46) is very efficient. For precise work with very small quantities, as occurs frequently in quantitative studies, a good form, sensitive to one-tenth of a milligram, is necessary, and excellent ones of this sort are sold by all dealers. The weights of these accurate balances must be carefully guarded against possible alteration through adherent grease, etc.

Photographic Outfit. This is essential in any modern scientific laboratory, but it differs so much with the taste and interests of the individual that recommendations are needless. My own includes a King Camera, with bellows extensible to permit photography of natural size, or even with a little enlargement. It takes a 5×8 plate, has a Zeiss Anastigmat lens, and is arranged on a solid tripod stand adjustable for height and angle. Directions for laboratory photography may be found in the volumes of the Journal of

Applied Microscopy; there is a useful note by WAUGH and MCFARLAND in the Botanical Gazette, 30, 1900, 204; and there is much valuable matter in SMITH'S "Bacteria in Relation to Plant Diseases" (Carnegie Institution, 1905). A series of white, black, and gray screens for backgrounds, arranged to work like roller shades, is very convenient. The most generally useful background for glassware is a sheet of blue blotting-paper.

METEOROLOGICAL RECORDING INSTRUMENTS. Of these a Thermograph for continuously recording temperature, and a Hygrograph for continuously recording humidity, may be considered essential, while a Barograph for recording barometric pressure has some, though relatively less, utility. These should stand under a proper shelter in the center of the experiment house (compare the photograph of Plate I). These instruments are very liable to work out of order, and hence should be standardized by comparison with accurate instruments (a good thermometer, a sling or cog psychrometer, and a mercury barometer respectively), at frequent intervals,—weekly when accurate work is in progress. The various standard forms of these instruments, together with the light recorders or Photometers, will be found described in a special section under Transpiration later in this book.

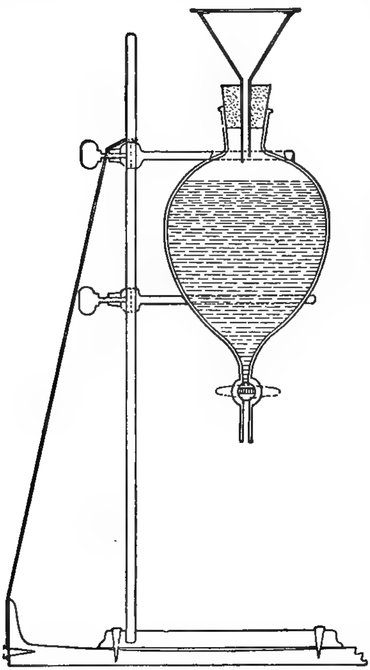


FIG. 9.—MERCURY CONTAINER; $\times \frac{1}{6}$. Supported in an indurated fiber saucer.

MERCURY CONTAINER, an essential feature of any laboratory. After trial of different forms, I have fixed upon, as most efficient and convenient, the arrangement figured herewith (Fig. 9). The mercury, made perfectly clean by some one of the methods described under Manipulation in Part III, is contained in a globular separatory funnel, of five inches diameter. From the lower end the mercury is drawn off through a stop-cock; and into its upper end the mercury is introduced through a funnel, allowing a final filtering through a pin-pricked paper. The funnel is supported by two iron rings of a chemical support-stand, the flat iron base of which rests in, and is firmly screwed to, a large (eighteen-inch diameter) indurated fiber saucer of the stoutest obtainable kind; the tendency of the heavy mercury to bend the support-rod is overcome by a strong wire passing from near the top of the latter diagonally outward and downward to a screw in the base of the saucer. The latter is provided with strong handles (not shown in the figure, as they are at right angles to the support), permitting the whole arrangement to be readily moved, though in fact it generally stands on one of the apparatus tables in the laboratory. A great advantage of the saucer is that it saves any spilled mercury, and would save all in the globe if this became broken, or if the stop-cock were carelessly left open. At the same time the saucer gives room for various accessories connected with the use of mercury, including the bottle for used mercury, which should never be returned to the container without cleaning. The saucer in the drawing is hollowed in the middle, but saucers can be obtained with the middle raised, in which case an opening, closed usually by a stopper, could be made at the lowest place near the margin, and thus the spilled mercury could readily be drawn off. A different method of preserving mercury clean and ready for use is described by ARTHUR in the Botanical Gazette, 22, 1896, 471.

PRESSURE-TESTING MANOMETER. It is often necessary to test the tightness of joints, stop-cocks, etc., to considerable gas-pressures, for which the arrangement figured herewith (Fig. 10) serves admirably. To the upper end of a piece of stout glass tubing, of about 5 mm. internal diameter and 75 cm. length, there is attached, by stout rubber tubing, a small funnel, while to its lower end there is attached a stop-cock joint of the kind, and bent to the form, shown by the figure. The free end of this latter piece is provided with a stout rubber tube into which may be inserted, and wired, the tube or other piece to be tested. When mercury is poured into the funnel, any desired pressure up to an atmosphere may be exerted upon the gas in the piece under test. The stop-cock permits this pressure to be released, and allows the apparatus to be drained of mercury, as may be desired. The whole arrangement is tightly wired, with rubber cushion pieces between, to a suitable board, which may be kept hung up when not needed, and may be stood in the mercury saucer, attached by clamps to the upright rod, when in use. The instrument can also be used to give an atmosphere of air-pressure for other purposes.

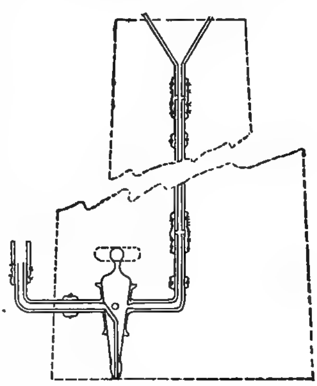


FIG. 10.—PRESSURE-TESTING MANOMETER, WITH SOME 70 CM. OF THE LENGTH OMITTED; $\times\frac{1}{4}$.

DRYING-OVEN. This is especially needed in the several studies where it is necessary to secure dry weights of tissues. Many forms are obtainable from dealers, one of the simplest and best of which is a double-walled copper box, with water between the walls heated by gas below; it has a side door, and openings in the top for exit of moisture, for thermometer, etc. I am now using with much satisfaction a new oven, made by the International Instrument Company of Cambridge, Mass., heated by the incandescent current acting upon resistance plates at the back. It has the advantage that it gives above 90° near the plates, and all gradations down to about 50° towards the door, while it is otherwise convenient and very neat in appearance. It does not, however, keep very constant in temperature. Of course any water-bath, even a beaker standing in a dish of warmed water, may be made to serve as a drying-oven.

Gas-generators. Those most needed are for carbon dioxide and for hydrogen. I like best the Kipp's automatic form, capacity 1 quart, sold by all supply companies. They are most convenient when kept, with their wash-bottles, upon a tray provided with handles, permitting their ready transport. If carbon dioxide in large quantities were needed, it would be better to use the commercial compressed gas now everywhere obtainable, and even the omnipresent seltzer bottle can be used at a pinch. Simple and efficient, though rather wasteful, generators can be adapted from common bottles with stoppers bored for an outlet tube, and for a thistle-tube through which the acid is poured in. But a much more economical form can be constructed from common laboratory materials exactly upon the Kipp's principle. The stopper of a wide-mouthed bottle is bored to take an outlet tube and the large end of a calcium-chloride tube, the bulb of which hangs near the bottom of the bottle, the whole arrangement being made tight by hard paraffin. The marble or zinc rests upon a paraffin disc attached to the calcium-chloride tube just below the bulb. When not in use the outlet is closed and the accumulating gas forces the acid into the calcium-chloride tube, which is loosely stoppered.

In addition to these larger, and more or less permanent, laboratory furnishings, there are many equally indispensable smaller articles which are not only needed for the daily manipulation of the laboratory, but are also desirable in anticipation of that constant trial for new and better methods, and of those special studies and investigations which ought constantly to be in progress. These are, of course, aside from the special appliances for particular experiments, all of which will be described in the appropriate places in Part II. The general appliances are the following:

Tools. Files, round and triangular, three or four sizes of each, and a large, flat one. Pliers, combined wire-cutting and twisting. Spirit-levels, small, including one circular. Soldering outfit. Carpenter's hammer, square, dividers, fine saw—in fact a small case of the standard tools of good quality. Small metal-working set. Vernier calipers. Whetstone. Scissors and shears. Set of cork-borers, and cork-presser. Small pruning shears.

Glassware. Bell jars, in pairs (for control experiments) of the different sizes, and several pairs of a standard size, preferably with ground neck and stopper and ground base; and ground-glass plates for the same. Also the standard forms of beakers, test-tubes, crystallizing dishes, conical flasks, thistle-tubes, wash-bottles, Petri dishes, burettes, cylindrical graduates (these latter 5, 25, 100, 500 cc. capacity, kept in a rack over the sink), funnels (say 5, 8, 13, and 20 cm. diameter, kept in a rack over the sink). Glass stop-cocks. Thermometers (two dozen of good grade chemical form, from 0° to 100°, with at least one carefully standardized form for comparison). Glass tubing (a full supply of all sizes, kept in drawers under the gas-table), especially 2 to 7 mm. inside diameter, with some barometer and some capillary. Sheets of white glass (cleaned negatives are excellent). White saucers and plates. Bottles of diverse forms and sizes. U tubes. Watch-crystals. Plain tumblers. Battery jars. Soyka flasks. Slides and covers.

Other Supplies. Cork stoppers, many sizes. Rubber stoppers, especially 20 to 32 mm. diameter at large end. Rubber tubing, various sizes, some black, some white (best bought fresh, as it spoils in time). Measures, wooden metric, to serve as scales on tubes. Electrician's tape (one of the most valuable materials of the laboratory, for making air-tight joints and for many other uses). Tin-foil. Stop-cock grease. Sealing-wax. Copper wire (sizes 16, 18, 20 are most useful; should preferably be on spools hung over the tool-table). Beeswax. Artists' modelling-clay. Vaseline. Plaster of Paris. Glue. Filter-paper. Cross-section paper (millimeter kind). Gummed labels. Tracing-linen. Black paper, preferably white on one side. Cheese-cloth. Pins. Strong thread. Porous flower-pots and saucers of different sizes. Pulpwood saucers, pairs of four or five sizes (may be obtained from dealers in gardeners' supplies). Supports and clamps of the ordinary chemical sort. Sphagnum moss may be obtained, if no local supply is available, from all dealers in gardeners' supplies. Blocks and wedges of wood for supporting apparatus. Hard paraffin (in a water-bath saucepan, always ready for safe heating).

Chemicals. All in suitable, well-stoppered and well-labeled bottles. Alcohol. Mercury. Formalin. Turpentine. Ammonia. The common acids. Salt. And the others mentioned in the following pages.

Seeds. These, of the much-used common kinds, Corn, Oats, Wheat, Barley, Sunflower, String Bean, Horse Bean, Lima Bean, Morning-glory, Garden Nasturtium, Squash, Castor Bean, Japanese Buckwheat, White Mustard, Radish, Lupine, should be kept in suitable bottles on a shelf of the chemical case. They should be obtained fresh each season from a reputable firm of seedsmen, the old stock being always thrown away just as soon as the quality of the new has been proven. Poor seed can vitiate an experiment to an extent unsurpassed by any other cause.

Many of the furnishings, apparatus, and supplies of the physiological laboratory, both those above described and others to be mentioned in the following pages, are standard articles of Physics and Chemistry, and can be bought from any dealers in those supplies. But of firms making a specialty of supplies for Plant Physiology, there are as yet but few, though there are some. In Europe the various appliances devised by specialists, mostly of a precision character, are made by various scattered individual "mechaniker," of whom the most important is Albrecht of Tübingen, who makes Pfeffer's best pieces. The apparatus recommended by Detmer is all supplied by Desaga of Heidelberg, and by Gallencamp & Co. of London, England. Various pieces of normal and precision apparatus, largely devised by Francis Darwin and his associates, are supplied by the Cambridge Scientific Instrument Company of Cambridge, England. In America there are four firms supplying instruments of this character. The Cambridge Botanical Supply Company of Cambridge, Mass., advertises many pieces which are in part identical with, or else modified from, those described in the first edition of this book, though made without any co-operation of mine. A second is J. C. Arthur of Purdue University, Lafayette, Ind., who has devised several excellent pieces (described mostly in the Botanical Gazette, **22**, 1896, 463), and has had them made for sale by the University mechanic. The third is the C. S. Stoelting Co. of Chicago, which offers a few good pieces, without mention of the name of the inventor, who is known, however, to be F. E. Clements. Finally there is the Bausch and Lomb Optical Company of Rochester, N. Y., which has undertaken the manufacture of such apparatus more extensively and systematically than any other firm. They make the various pieces of my own normal apparatus, and attempt to supply all other materials and articles needed in Plant Physiology, of which apparatus and supplies they issue a special catalogue.

PART II.

A COURSE IN EXPERIMENTAL PLANT PHYSIOLOGY.

THE PHYSIOLOGY OF PLANTS.

PHYSIOLOGY is the study of the activities of living matter. It must therefore take account of the structure and properties of the living matter, called Protoplasm, as well as of the processes which it carries on. Hence the Physiology of Plants falls for study somewhat naturally into divisions as follows:

Division I. The Structure and Properties of the Protoplasm of Plants.

Division II. The Physiological Processes of Plants.

DIVISION I.

THE STRUCTURE AND PROPERTIES OF THE PROTOPLASM OF PLANTS.

PROTOPLASM, like every other substance, exhibits a distinctive physico-chemical structure, composition, and properties. But in addition it possesses also some unique qualities correlated with its vital activities, and these involve remarkable relations with the external world, including the important power of building organisms. Hence the study of this division of Physiology falls rather readily into sections as follows:

Section 1. The physical structure and properties of Protoplasm.

Section 2. The chemical composition of Protoplasm.

Section 3. The vital structure and properties of Protoplasm.

Section 4. The reactions of Protoplasm to external forces.

Section 5. The building of organisms by Protoplasm.

SECTION 1. THE PHYSICAL STRUCTURE AND PROPERTIES OF PROTOPLASM.

A knowledge of this subject requires, first of all, an exact observational study of living Protoplasm from favorable plant sources. Thus is presented a definite inquiry as follows:

What physical structure and properties, as manifest in optical qualities, in texture, in density, in motility, and in differentiations of substance, are visible to exact observation under favorable conditions in the living Protoplasm of plant cells?

Since Protoplasm exists in most plants only in minute and thinly distributed masses, it is necessary to resort to the aid of the microscope.

OBSERVATION. Select the best of the available materials showing living Protoplasm; mount each in tap-water on a slide under a cover-glass; examine carefully with a microscope, first with lower powers, and then with the highest at command. The results of the study should be expressed in such a combination of drawing and words as will most accurately and vividly bring the facts before the mind of a reader.

MATERIALS. The best, because most clearly visible, material occurs in transparent-walled, thread-like, or hair-like structures. First among these are the hairs on the stamens of the *Tradescantia virginiana*, or *Virginica* (Spiderwort), in any of its several varieties, of which var. *pilosa* of gardens is very good. The hairs should be taken, grasped with fine forceps by their bases, from just opening or even still unopened blossoms. This plant ordinarily blooms in gardens in May and June, but may readily be obtained in perfect condition from September to December by planting in a cold frame or greenhouse and cutting it back near to the ground just before it blossoms in spring. Other species of *Tradescantia*, especially *T. zebrina* (Wandering Jew), commonly grown in greenhouses, yield hairs nearly as good. A fair substitute is offered by the hairs on the stems and leaves of young Squash plants, which may be grown at any time in pots from seed, requiring only two weeks for sufficient development, while other *Cucurbitaceæ* also afford good hairs. Other plant hairs, such as those from *Cypripedium spectabile* (Lady's Slipper), are also available. Many root-hairs, grown in germinators, the mycelia of some Fungi, and transparent-walled pollen-grains, especially if they form tubes, or even burst in the water, are also excellent. Of great value are the Stoneworts, species of *Chara* and *Nitella*, especially the latter; these are found nearly everywhere in ponds and slow streams. If brought into a greenhouse, allowed to root in a layer of mud at the bottom of a tub, and kept in a moderately lighted place, they will continue to form the young tips, which are best for this purpose, during several weeks. In older cells the Protoplasm is greatly obscured by the chlorophyll grains, but young tips may be found in which it is perfectly clear. With this, as with other materials in which protoplasmic motion exists, the presence of the motion can be taken as evidence that the Protoplasm is in normal condition. Growing in somewhat similar situations, and capable of greenhouse cultivation in the same way, is the Water-weed, *Elodea*, or *Anacharis, canadensis*. The plasmodia of slime-moulds, *Myxomycetes*, may sometimes be found on decaying wood in damp, dark places, and are among the best of materials, especially when made to grow upon slides. Additional practical directions for the examination of these objects, and as well some reference to other good materials, may be found in Chapter 3 of STRASBURGER'S "Handbook of Practical Botany" (translation by HILLHOUSE, New York, Macmillan Co.). So far as materials for the study of movement are concerned, there is a thorough study of the subject from the present point of view by GRACE BUSHEE in the Botanical Gazette, **46**, No. 1, 1908. In this paper a list of available materials, including all of those above mentioned, together with many additional greenhouse plants not heretofore recorded, is given, along with their rates of movement. Among the best materials, in addition to those before mentioned, are the hairs from *Abutilon*, Tomato, *Gloxinia speciosa* (especially good), *Whitlavia grandiflora, Lobelia Erinus*, Heliotrope, and *Pelargonium* species.

DEMONSTRATION. It is possible to project the Protoplasm upon a screen so as to make its structure (in part), and even its movement, visible to classes of considerable size; but this requires a fortunate combination of projecting microscope, excellent material (preferably *Nitella*), and careful preliminary preparation. Some directions therefor are given by PFEFFER in his paper earlier cited (p. 23, note).

Of the various features displayed by the living Protoplasm studied under the preceding observations, the most striking is its movement, called *Streaming*. So remarkable and apparently characteristic is it that it calls for more exact study, which involves the following inquiry:

What is the character of the streaming in living Protoplasm, as to amount of the Protoplasm involved, constancy of path and direction, place of greatest activity, and rate under normal conditions?

This requires accurate microscopical study, with the three-dimensional nature of the movement in mind, together with microscopical measurements.

OBSERVATION. Select the most actively streaming of the available materials in the foregoing list, preferably *Nitella* and *Tradescantia*, mount them in water for the microscope, and determine the observable facts about the movement. Then determine by direct or by micrometer measurements, reduced to millimeters per minute, the rate of the swiftest streaming at the room temperature, which is to be noted. The results should be expressed not only in words and figures, but in drawings representing diagrammatically in all three dimensions the paths and extent of the streaming.

PRECAUTIONS. To ensure normal streaming, one must take care not to give the material any sudden shock, either through rough handling, injury by forceps, pressure on cover-glass, or sudden transitions in temperature. It is best to mount the material and let it stand for some time, a quarter of an hour or more, before using, in the case of *Nitella*, the material, if prevented from drying up by use of a small moist-chamber made with filter-paper and a tight glass dish, may be kept in perfect condition for hours or even days. Enough water must be used in the mount to float up the cover-glass and prevent it from pressing on the specimen. Observation of the first specimen seen is to be shunned; a number should be looked over, and typical ones, preferably several, should be selected, and the mean of their rates determined. The lowest magnification and longest spaces of movement practicable give most accurate results. Other precautions are given in EWART'S "Protoplasmic Streaming," 24, 53; and the student should especially be on guard against the various sources of error, as discussed earlier (page 14) in this book.

MICROSCOPICAL MEASUREMENTS. These can be made approximately by determining first, with a stage micrometer, the diameter of the field of vision, and then noting the time requisite for certain granules to cross a given fraction of the field. But far more accuracy is attained by the use of an ocular micrometer (a scale ruled on a glass disc which can be placed on the diaphragm of the eyepiece), which is clearly in focus with the moving Protoplasm. The value of the spaces in millimeters depends upon the magnifying power of the combination with which it is used, and while this may be stated by the maker, it is much better to determine it anew for each combination by comparison with a stage micrometer ruled in small fractions of a millimeter. Then the time requisite for streaming a given distance is readily determined. The time may be counted by a watch or a clock; but far better is a second-ticking clock or a metronome, the inexpensive instrument used in musical instruction. For very exact work a stop-watch would no doubt be best of all.

PROTOPLASMIC-STREAMING QUANTITIES. The rate of streaming varies from near 0 to 10 mm. per minute, though the latter is very exceptional; for most plants the optimum rate lies between .3 and 1 mm. per minute, and may conventionally (page 22) be taken as .7, thus making the conventional expression 0–.7–10 mm. per minute. The rate is correlated with temperature, rising from a minimum degree of no movement up to an optimum degree of greatest rate, whence it sinks again to a maximum degree of no movement. These three points vary with different plants, but approximate to, and may conventionally be expressed as, 5°, 35°, and 45°.

Graphs showing the relation of temperature to rate of streaming are accessible for *Nitella* in this book at page 21, and for *Chara*, *Vallisneria*, and *Elodea* in DAVENPORT'S "Experimental Morphology," 226.

The student should now, calling the literature to aid, ascertain what is known as to *classification of the forms of movement, how widely it prevails through living cells, what is known or (more properly) what is supposed as to its meaning to the plant, and what its physical basis (energetics) is.* The latter matters he can best follow through the works of EWART and BÜTSCHLI, and he should know something of the artificial foams, developed by the latter, which simulate the protoplasmic movements.

During the foregoing study the student should take note of any appearances which testify to the ultimate mechanical structure of the Protoplasm,—whether this is a finely fibrous mass soaked with liquids, as the older views supposed, or an emulsion of liquids holding solid bodies in suspension, as the newer conception, especially advocated by BÜTSCHLI, maintains. It is possible this problem will be solved by the ultraviolet microscope, a very new instrument using such short light waves as to give clear definition to extremely minute objects; and the student should follow henceforth the developments in this line of investigation.

There is another method yielding knowledge of protoplasmic structure, *viz.*, the killing and staining of the substance by the methods used in histology, especially in cytology. The student should make himself acquainted, preferably through study of cytological preparations, with the structures thus shown to exist in the dead Protoplasm, and with the probabilities as to their existence in Protoplasm while alive. The details of cytological phenomena are, from the present point of view, obviously not important.

The study of any, even of all, of the above-mentioned materials gives a knowledge of living Protoplasm, which, while accurate, is yet very limited, and it should receive extension by further study. This should be observational, if that be practically possible, and literary if it be not, and should embrace a wider range of materials, representing the substance under more special, and even extreme, conditions, such as in cells that are growing, reproducing, storing, synthesizing, resting, etc. Also the nature of animal Protoplasm and its relations with that of Plants should be included.

LITERATURE. The most important works for the student upon this subject are VERWORN'S "General Physiology," and the summary in PFEFFER'S "Physiology," and in JOST'S "Lectures." Valuable also, especially for discussion of protoplasmic structure, is WILSON'S "The Cell in Development and Inheritance" (New York, Macmillan, 1904), and there is an excellent summary article by him in Science, **10**, 1899, 33. On the emulsion structure of Protoplasm, upon its movement and the imitation thereof by artificial microscopic foams, there is the important work of BÜTSCHLI, translated as "Protoplasm and Microscopic Foams" (London, Black, 1894), of which descriptive reviews are in Science, **2**, 1895, 893, and in Nature, **48**, 1893, 594. Of fundamental importance for protoplasmic movement is EWART'S fine monograph, "Protoplasmic Streaming in Plants" (Oxford, Clarendon Press, 1903). But it is of interest to note that the existence of the emulsion structure in living plant cells has recently been denied by DEGEN in Botanische Zeitung, **63**, 1905, 202.

SECTION 2. THE CHEMICAL COMPOSITION OF PROTOPLASM.

Protoplasm has been found to be, chemically, not a single substance, but a mixture of many and diverse substances, including some which are very complex and unstable. It is thereby rendered very difficult for chemical study, to such a degree that it is still doubtful whether there exists a distinctive chemical Protoplasm to which the other constituents are secondary, or whether it is simply a mixture without any distinctive life-constituent. The practical chemical study of Protoplasm requires methods and appliances so special as to make it a difficult phase of organic chemistry, and it is not profitable for the student to undertake such work as a part of this course. But he should make himself acquainted through books with our present knowledge of *the chemical composition of Protoplasm, including the composition of those important protoplasmic constituents, such as nuclein and plastin, whose composition is known, the chemical* *lability of the proteid constituents, and the probable relation of that lability to the phenomena of life.* Certain related matters, such as the chemical stages in the formation of Protoplasm, and its mode of releasing energy for the performance of work, can be treated more profitably later under Conversion and Respiration.

LITERATURE. Upon this subject knowledge has not advanced materially in recent years, and little has been added to the work of REINKE and RODEWALD in 1881–1883, which may be traced through PFEFFER'S "Physiology," and the chemical results of which are given in brief in JOST'S "Lectures" and in GOODALE'S "Vegetable Physiology." The subject is treated fully and technically in CZAPEK'S recent exhaustive work, "Biochemie der Pflanzen" (Jena, Fischer, 1905, two vols.). On the relations of chemical composition and lability to vital activity, there is a short book by LOEW, "The Energy of Living Protoplasm" (London, Kegan Paul, 1896), and there is a good synoptical article by the same author in Science, **11**, 1900, 930. Of most importance on this subject, however, is LOEB'S book, "The Dynamics of Living Matter" (New York, Columbia University, 1906), to which should be added his article on the "Chemical Character of the Process of Fertilization" in Science, **26**, 1907, 425.

SECTION 3. THE VITAL STRUCTURE AND PROPERTIES OF PROTOPLASM.

Living Protoplasm, as already noted, is unlike all other known substances in possessing two sets of properties or qualities,—a physical set already studied and a vital set here to be considered. It is indubitable that in their ultimate nature the two are not different in kind, and the vital will sometime be found analyzable into the physical. Nevertheless the vital properties are not at present resolvable into anything simpler, nor are they known to exist outside of the living organism, for which reasons it is logical as well as convenient to consider them apart. The student will gain a knowledge of these properties during the work of the present course, and it will here suffice, in order to complete the present topic, merely to name them. They are variously classifiable, but may be designated thus: *metabolism*, *growth*, *division*, *motility*, *responsiveness to stimuli*, *regulation*. A feature which distinguishes all of these from physical properties is this: they are not static, but dynamic, and, moreover, are independently, internally, spontaneously, or auto dynamic. They might, therefore, be more properly described as *powers*, rather than as properties. It is the manifestations of their activity in the substance forming their physical seat which constitute the phenomena of life; and it is their interaction with the kinetic forces of external nature which develops organic structure and habit. If it shall ultimately be found that there is any feature in Protoplasm not existent in inorganic nature, it is probably connected with the power of self-regulation, which is the most remarkable and distinctive of all the attributes of the living substance.

The physical properties of any substance, Protoplasm included, are believed to reside in its molecules, which are the units of physical structure. It is not logically conceivable that in Protoplasm both the physical and the vital properties reside in the same units, and hence investigators have had to assume also a unit of vital structure (*plasom*, etc.), presumably larger than the molecules and aggregates of them, a subject with which the student should make himself acquainted through the literature. Incidentally it is noteworthy that this logical necessity involves the assumption of a distinctive chemical Protoplasm.

Correlated with this subject is the consideration of (*a*) the phylogenetic origin of Protoplasm, a matter on which we have no knowledge and little rational speculation; (*b*) spontaneous generation; (*c*) the distribution of Protoplasm in space and range of temperature. Upon the state of knowledge of these matters the student should also inform himself.

LITERATURE. The subjects of this section are comprehensively treated in VERWORN'S "General Physiology" and in PFEFFER'S "Physiology." There is a classification of vital properties in the chapter introductory to VINES' "Lectures." The most thorough dicussion of the units of protoplasmic structure and properties is WIESNER'S "Die Elementarstructur und das Wachsthum der lebenden Substanz" (Wien, 1892).

SECTION 4. THE REACTIONS OF PROTOPLASM TO EXTERNAL FORCES.

Living Protoplasm is of soft and plastic consistency, contains many complex and highly unstable constituents, and is the seat of numerous and diverse energy changes. It is thus brought into extremely delicate and unstable touch with the innumerable forces acting upon it from the environment, and the resultant relations are of the utmost physiological importance. Theoretically it might seem best to study such relations by direct microscopic observation of the effects of the forces upon the living Protoplasm, but with rare exceptions this method entails great or insuperable manipulative difficulties. Practically, therefore, it is best to infer the relations from the action of the forces upon entire plants, and in this way the student will gradually become acquainted with them during the present course. It must suffice to consider under this section certain typical cases in which the reaction of protoplasmic activity to external forces can be directly observed. These forces are essentially these six: *Heat, Light, Electricity, Mechanical Impact, Chemical Action, Gravitation.* As to their action upon the Protoplasm, it is plain, both upon theoretical grounds and also from the results of experiments with which the student will later become familiar, that they may operate in either one of three ways, which ways are recognizable for practical convenience as distinct, though ultimately they are no doubt related if not identical.

First of the three ways, the forces may act upon the Protoplasm purely mechanically, physically, or chemically, precisely as they would upon any other substance of similar constitution, quite regardless of its vital properties, or whether it be living or dead. Thus heat may coagulate it or burn it to ash; pressure may crush it; a chemical may dissolve it, and gravity may pull its heavier down through its lighter parts. Here the force must be present in considerable amount, its action is direct or immediate, the result is proportional to its intensity, no question of advantage or disadvantage to the organism is involved, and the Protoplasm is purely passive and responseless. The study of such direct relations belongs rather to physics and to chemistry than to physiology, and, in their simpler manifestations, they are usually somewhat obvious. Yet, if the student have time and the will to observe some of them for himself, he may do so through the following:

SUGGESTED EXPERIMENTS. For these the living Protoplasm is selected as before and mounted for the microscope.

Heat. Place the slide on a metal plate perforated under the objective and clamped, with an intervening thick felt or cloth, to the microscope stage; apply heat to a projecting part with a spirit-lamp, and observe the Protoplasm through the various changes to disintegration.

Mechanical Effects. Apply pressure with needles upon the cover-glass, and observe the disorganization effects up to complete destruction.

Chemical Action. Apply upon one side of the cover-glass a drop of 10% solution of caustic potash; draw it under by filter-paper applied at the opposite margin; continue from time to time, and observe the result upon the Protoplasm.

Gravitation. Select material, such as *Tradescantia*, which has a conspicuous nucleus, and mount it so that when the microscope is set horizontally the nuclei will be at the upper ends of the cells; from time to time note the result upon their positions.

Or mount a streaming cell of *Nitella* after the manner described by EWART, 23–25, so that it will be vertical when the microscope is horizontal; observe, by aid of measurement, whether the more solid particles move at the same rate up and down.

Electricity. Arrange, by the method described on page 71, a streaming cell, so that its ends are in contact with tin-foil strips which can be thrown into circuit with 1, 2, 3, or 4 dry battery cells, and note effects upon the Protoplasm. Test also, if practicable, the effects of an equivalent induced current.

Light. Prepare a slide of some material which develops naturally in darkness or in dim light, *e.g.*, a root-hair, and, taking precautions to prevent action of heat (Part III), focus upon it the fullest possible obtainable light, and note result. Here consider the germicidal power of light and its supposed physico-chemical basis.

The second of the three ways in which external forces may act upon Protoplasm happens to be illustrated with great clearness by the action of heat upon protoplasmic streaming, a matter which should now receive careful experimental study.

What effect is produced upon protoplasmic streaming by heat in its different degrees?

For this it is needful to use some method by which the temperature of the living Protoplasm can be raised and lowered at will, while the rate of movement is measured at each degree.

EXPERIMENT. Select the best of the available materials showing protoplasmic streaming, preferably *Nitella* or *Chara*, and mount as for the preceding studies. Then place the slide in position on a temperature stage, and slowly raise the temperature, determining the rate of streaming at regular intervals until it ceases. Then take another specimen and lower the temperature, measuring as before until streaming ceases.

Again, for a more accurate determination wholly from the same individual specimen, prepare good material as before, and first cool the stage to zero. Then slowly raise the temperature through the entire course of the movement, measuring as before. Plot the result in a polygon designed to exhibit graphically the relation of rate of movement to temperature.

PRECAUTIONS. Those given for the study on page 60 should be carefully observed. The raising and lowering of the temperature must be slow, not faster than a degree in two minutes, else the change acts as a shock to affect the movement. Care must be taken to keep the specimen from drying out, which can be managed by giving it abundance of water at the start; if needful to add it later, the water must be of the same temperature as the specimen, and preferably taken from a small vial kept for the purpose on the stage. The flat mirror, without a condenser, should be used, to minimize the focusing of heat with the light. It is difficult to measure the rate for every degree, and practically it suffices to measure it at alternate degrees, preferably the even numbers to facilitate comparison of results.

TEMPERATURE STAGES. Of these there are many forms, the chief of which are mentioned below. The requisites of a good stage are these: that its temperature can be readily carried under perfect control from below zero to at least 60°, and that thermometer and object are always at the same temperature. According to whether the latter is effected perfectly in principle, or only approximately, they divide into grades from precision downwards.

Of precision forms the first seems to have been NÄGELI'S (1867, in NÄGELI and SCHWENDENER, "Das Mikroskop," described by VELTEN in work cited below in Literature), in which the object and thermometer are completely surrounded by flowing water of known temperature; but it is elaborate and does not admit use of high powers. Then followed the STRICKER electric-heated stage (1871, described by VELTEN), which is also elaborate. Then SACHS developed his microscope chamber (1873, figured in his later Text-books, and by DETMER, 421), which was a double-walled box almost completely enclosing the microscope, with a window for light and small openings for manipulation of the slide; it was warmed by heating water between the walls from a lamp beneath, and cooled by ice and salt between the walls. Much improved in details by various workers, it may now be bought in several forms, under the name Microscope Thermostat, made by ROHRBECK of Berlin. It is, however, not readily cooled, and is better adapted for keeping constant temperatures than for changing them. Very simple was the arrangement used by VELTEN (1876), consisting of a crystallizing dish of water, which can be cooled or warmed by flowing water, and in which, wholly immersed, was the object, the objective, and part of the thermometer tube; but it required the use of the now rare water-immersion lens. PFEFFER (Zeitschrift für wissenschaftliche Mikroskopie, **7**, 1890, 433) has used it with improved details. Theoretically an efficient form could be constructed on the principle that object and thermometer bulb should lie side by side, embedded in suitable openings in the same glass plate and surrounded by one continuous film of water, but such apparatus is very difficult to manufacture.

Of normal forms by far the best is that made by VÉRICK (figured by GOODALE, 202), a double-walled box surrounding a place for the slide and heated by circulation of water from a reservoir; thermometer and object being in separate compartments, their temperatures need not be the same, though in fact they agree so nearly as to make this stage in practice a precision instrument. More or less modified, it has been used by EWART (60) and others, and is sold by various makers in a variety of forms, most of them taking the slide not in a chamber, but upon the surface, *e.g.*, the STRICKER form. Far less accurate is the SCHULTZE form (1865, figured in VERWORN, 392), consisting of a flat metal plate with a thermometer in contact; it permits a considerable difference in temperature between object and thermometer, and cannot readily be cooled. I have myself devised a form which is amply accurate and convenient for student use (Botanical Gazette, **27**, 1899, 257); somewhat improved, it is among my normal apparatus (page 46) and is figured herewith (Fig. 11). It is made from a single sheet of copper, brought to the form shown by the figure; the prepared slide is placed in the flat chamber, where it can be moved about by the right thumb until the object is in position, when it is lighted from below through openings left for the purpose. The thermometer is placed with its bulb near the middle of the cylindrical chamber, and should sag a little so the bulb may press against the metal. To heat the stage, water is placed in the box and warmed by a spirit-lamp slowly moved in from the tip, and to cool it, broken ice, and later salt, are slowly added in place of the water. The stage is firmly clamped to the microscope stage with a non-conducting felt mat intervening. To reduce the temperature of the object below zero, it is necessary to place the microscope in a cool place, or, better, to enclose the stage in a tight-fitting, non-conducting, cloth cover. If properly used, the temperature variation between thermometer and object should not exceed 1° to 2°, and this may be reduced in using low powers by covering the chamber with a cover-glass, the proximity of the radiating or absorbing objective being the greatest cause of temperature error. Other precautions already mentioned above should be observed in its use.

Stages may readily be made to order in some such form as that described above (Botanical Gazette, 27, 1899, 256, and first edition of this book, 56). More simply (and necessarily less accurate) they can be made from a flat plate of copper having a sleeve for the thermometer, as described by E. L.

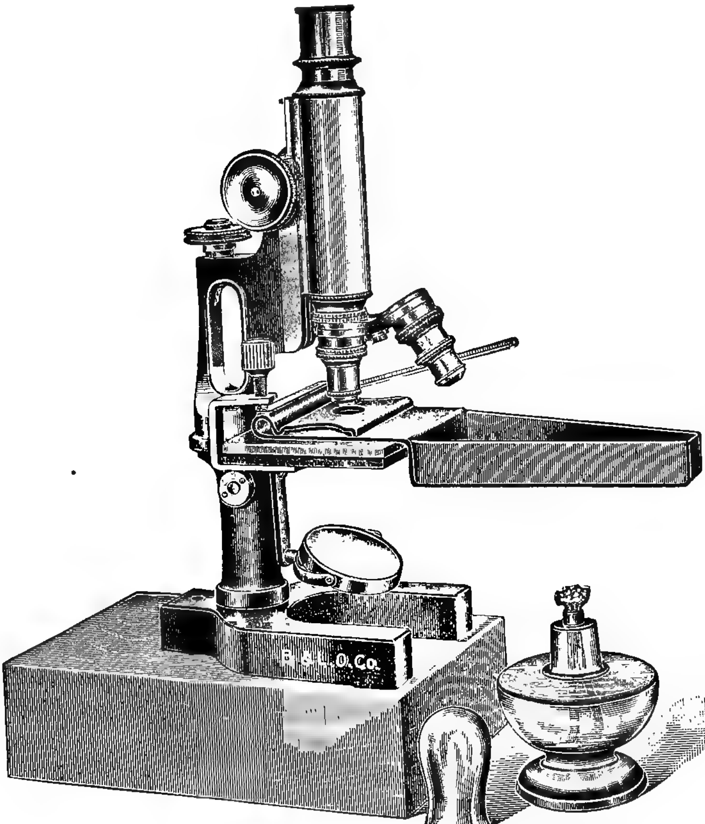


Fig. 11.—Temperature stage, student form; $\times\frac{2}{7}$.

Walker in the Journal of Applied Microscopy, 6, 1903, p. 2549. And they might even be improvised from a metal plate with thermometer clamped thereto, though results could be only of the crudest.

In this, as in all such studies where the manipulation is new to him, and where the individual variability of the material may also play a part, the student should not trust to a single experiment, but should try at least two, preferably more, and if possible ten, and should take the mean of the results. It will also be very advantageous for him to arrange with his fellow students to use uniform source and treatment of material throughout, so that the results may be compared and averaged to a single record which will have some value as a standard of comparison for individual results. And he should be constantly on the search for the detection and elimination of the sources of error (page 14). This entire study is well worthy the best efforts of the student, partly because of the clearness with which it illustrates this kind of physiological relations, and partly because of the unusually favorable opportunity it offers for training in the application of precise physical measurements to a physiological phenomenon.

The foregoing experiment, with its clear exhibition of a minimum point of no movement, a rise towards an optimum point of highest movement, and a fall to a maximum point of no movement, all under a steadily increasing temperature, affords an ideal example of this second kind of relation between Protoplasm and external forces. And the principle is the same even in those cases in which the force acts only to reduce movement. Such knowledge as we possess seems to show that the forces produce the results not directly or immediately, but indirectly or mediately, by promoting or hindering some of the dominant physico-chemical processes going on in the Protoplasm. Here the forces, present in moderate amount, act mediately or indirectly, the results are proportional to their intensities though only within certain limits, and the sum-total of the interactions of them all is the minimum-optimum-maximum result in the Protoplasm as a whole. Theoretically and primarily the results are independent of advantage or disadvantage to the organism, but in fact organisms are so adjusted to the more common of these forces, that the forces act to keep them in an advantageous condition of tone, whence the relation is designated as tonic.

As noted above, the three cardinal points, minimum, optimum, and maximum, are very characteristic of this tonic relation. Above the maximum or below the minimum, for an interval of a few degrees, as the student should confirm for himself, the Protoplasm remains quiet, but can have its motion restored by dropping or raising, respectively, the temperature. This condition of quiescence is known as rigor, and is also characteristic of differential relations. Above or below the rigor temperatures disorganization of the Protoplasm ensues, with death, the heat then acting upon it directly in the first of the ways above given.

The cardinal points have been found, experimentally, to be not fixed for each organism, but alterable by gradual accommodation to higher or lower temperatures. This is the principal physiological basis of acclimatization, a subject on which the student should here inform himself.

The foregoing case of a thermotonic relation is the clearest and most complete example of this kind of relation which can actually be seen in operation. The action of most others can in practice best be inferred from the results upon entire plants, as the student will find during his later studies. Yet there are some other cases which the student may observe if his time permits.

SUGGESTED EXPERIMENTS. In all cases streaming Protoplasm mounted for the microscope, as in the earlier experiments, is to be used.

Light. For this it is needful so to arrange that light in various degrees, from darkness to full sunlight, can be thrown at will on the streaming Protoplasm, the rate of movement being determined at the various intensities. It is essential that the influence of heat be eliminated, which can be done by avoiding concave mirrors and condensers, and by interposition of an alum bath or a stream of running water, for which purpose the gas-chambers, described below, may be employed. A control should be provided by placing a thermometer bulb in place of the object on a neighboring and similarly treated microscope.

Electricity. For the exact study, involving measurement, of this relation, somewhat elaborate and special appliances, including batteries, induction coils, non-polarizable electrodes, electrometers, etc., are needed, which, with the appropriate methods, are described in DAVENPORT'S, in DETMER'S, and especially in EWART'S works. But for qualitative results, sufficient for the present purpose, it may be simply studied as follows. On an electrical slide (described below), in contact with tin-foil terminals, place a *Nitella* or *Tradescantia* filament in active streaming, and then, with aid of a simple circuit closer, send through it the charge of successively one, two, and three dry cells, applying the current instantaneously (as nearly as possible), one, two, three, five, and ten seconds, and noting whether the making and breaking of the circuit or the continuous action of the current produces the result. It is well also to use an interrupted current through an induction coil, of which the DuBois Raymond form, permitting a large range with every intermediate gradation, is best.

Mechanical Impacts. These may be of several sorts, including sudden shock, pressure, alterations in atmospheric pressure, and variations of osmotic pressure. As simplest and most typical, we may take the first. For its study some method is needed by which the intensity of the shock may be measured. This is well accomplished by Ewart's method (73), in which a solid object, such as a knitting-needle, is dropped through a glass tube upon the cover-glass from successively greater measured distances. For study of the effects of pressure, a simple arrangement permitting the application of a screw to the cover-glass is possible. For variations in atmospheric pressure, the gas-chamber described below can be adapted, the material being placed in a drop of water hanging beneath the cover-glass, which is sealed (by sealing-wax) to the chamber. The pressure is lessened by a Chapman exhaust. Variations in osmotic pressure are easily observed by use of solutions of sugar or salt applied precisely as for the plasmolytic studies given later in this book under Absorption.

Chemical Action. Of the chemical substances exerting a tonic effect, three of the most important are oxygen, carbon dioxide, and anæsthetics. For the application of these it is needful to use a gas-chamber (described below), into which the gas can be drawn at will and thus applied to the object which hangs in a drop of water beneath the cover-glass. For the practical directions in detail the student should turn to the works of Detmer, of Darwin and Acton, or of Davenport.

Electric Slides. These are very simple in principle, consisting of an ordinary glass (and therefore self-insulating) slide, near the ends of which are attached small binding-screws in contact with metal clips extending near the cover-glass. From the clips tin-foil strips lead to the two ends of the object, through which a current may thus be sent. Such slides are sold by most dealers. The electrodes are obviously not unpolarizable, as they must be for exact work, on which the works of Detmer or of Ewart should be consulted. An efficient slide can be made up as shown by the accompanying figure (Fig. 12), in which simple binding-screws are attached to the slide by sealing-wax or by asphalt varnish. Or a perfectly good slide can be improvised from the ordinary clips of the microscope stage, which need only to be insulated in their sockets by rubber sheeting and then swung near the cover-glass, the wires being attached to the screws of the clips.

Gas-stages. These consist of flat chambers, partly or wholly of glass, made to lie on the microscope stage, and having inlet and outlet tubes through which the desired gases may be drawn by an aspirator. The object usually hangs in a water-drop on the under side of a cover-glass, which forms the roof of the chamber and is sealed thereto by soft wax; the water-drop is prevented from drying up by a little water in the chamber. The earliest and best form was ENGELMANN'S (figured by VERWORN, 520). A simpler form was introduced by PFEFFER ("Physiology," 3, 338); it can be bought from various dealers, who also supply, especially for bacteriological purposes,

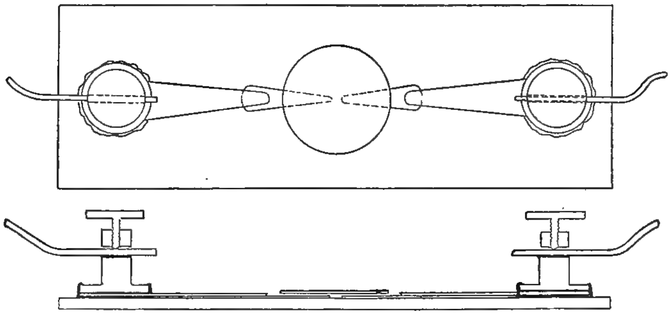


FIG. 12.—ELECTRIC SLIDE, ADAPTED FROM COMMON MATERIALS; ×½.
View of upper surface and vertical section.

still simpler forms. An efficient gas-stage can be adapted from strips of window-glass, glass tubing, and glue, with a coating of collodion to protect the latter from moisture; the construction is sufficiently illustrated by the accompanying figure (Fig. 13).

In the study of the above relations the student must guard against preconceived expectations, and keep his mind open for results as they are, even when these are negative. In one of these relations, the electrotonic, we are dealing with a kind which never occurs naturally. The observed response, therefore, cannot be explained upon any basis of adaptation, but must have another meaning. Correlated with these relations is the effect produced upon protoplasmic activity by the presence of more or less (down to practically no) water, with the very important ecological consequences; and on this subject the student should seek information in the literature.

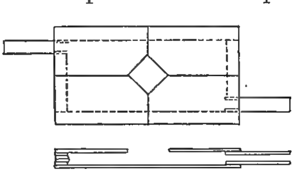


FIG. 13.—GAS-CHAMBER, ADAPTED FROM GLASS PLATES AND TUBES; ×½.
View of upper surface, with bottom or interior dotted, and vertical section.

We now resume our consideration of the different ways in which external forces may act upon Protoplasm. Two of these have already been considered.

Third of the ways is this: the forces so act that the Protoplasm moves (or bends) towards, from, or across their line of action. A familiar case of this is the bending of stems towards light, of leaves across it, and of roots from it, and there are innumerable others, some of which the student will later study in detail. Here the force acts like a signal, and there is an active response determined by internal factors of a nature at present quite unknown, though it follows from the existence of positive, negative, and lateral responses to the same force that the relation between force and response cannot be either direct or tonic. This relation is only very remotely quantitative, as will be shown later in this course (indeed, a notable feature of this relation is the smallness of the stimulus necessary to produce a great result), and it usually, or always, involves advantage to the organism. The Protoplasm is said to be *irritable* to the force, which is said to act as a *stimulus*, though the latter word is also used, unfortunately as I think, for forces acting tonically, and even sometimes, though certainly erroneously, for forces acting directly. In a general way the protoplasmic response is direct to a considerable intensity of a force, tonic to an amount too small to produce a direct response, and irritable to an amount too small to produce a tonic response; and through this curious relationship the real nature of irritable responses may yet be elucidated.

The action of stimuli (of this third relation) does not produce any effect upon the streaming of Protoplasm or upon any other visible feature, aside from certain movements of locomotion of chlorophyl grains, motile bacteria, myxomycetes, or zoöspores. Hence it is nearly always much easier to infer the processes from the actions of entire plants than to observe them in the Protoplasm. Accordingly the student may here best leave these matters until he takes up the subject of Irritability, where they will all be fully considered.

LITERATURE. On this subject there is an immense literature, especially on Irritability; it will be considered later under that subject, and here we need only note the most general papers, which, in addition to the works of PFEFFER, JOST, and VERWORN, are the following. On streaming of Protoplasm there is the admirable monograph by EWART, "Protoplasmic Streaming in Plants" (Oxford, Clarendon Press, 1903), which brings the subject so nearly to the present date that I have since noted only a single paper of importance, SCHRÖTER on streaming in moulds in Flora, **95**, Ergänzungsband, 1905, 1. An older paper which will repay study is VELTEN'S in Flora, **34**, 1876. An admirable summary of all the literature upon the general subject of the effects of external conditions upon Protoplasm is in Part I of DAVENPORT'S "Experimental Morphology."

SECTION 5. THE BUILDING OF ORGANISMS BY PROTOPLASM.

In these studies so far Protoplasm has been studied simply as a unit without regard to any organization it may exhibit. Its masses, however, are not formless, but are highly differentiated in structure, presumably in correlation with function. The subject is one for study by the anatomist, histologist, and cytologist, as well as by the physiologist, but its physiological importance here demands some unified consideration which, necessarily, must be rather theoretical than practical. For completeness of the subject, and as a basis for future studies, the student should inform himself upon the state of knowledge of its important phases, which are included under the following headings:

(*a*) The differentiation of Protoplasm into specialized structures, cytoplasm, nucleus, plastids, with vacuoles and wall; the known or supposed significance of the division and functions of the parts. (*b*) The division into protoplasts or cells, and its meaning; sizes of the cells and the determinants; method of the division and its meaning. (*c*) Construction of the skeleton; the cell wall and its substance, with the composition of the latter and its derivatives; the diverse cell shapes and their meanings. (*d*) The physiological continuity of the different protoplasts and the mode of its maintenance, with any special structures to this end. (*e*) The development of tissues and of organs.

LITERATURE. For this the student would turn to the appropriate sections in PFEFFER'S "Physiology." The great work upon the physiological and ecological phases of structure is HABERLANDT'S "Physiologische Pflanzenanatomie." The subject of cell size and its determinants is discussed fully by SACHS in his "Physiologische Notizen," in Flora, **77**, 1893, 49. Upon protoplasmic continuity the latest and best work is by STRASBURGER in Jahrbücher für wissenschaftliche Botanik, **36**, 1901, 493.

DIVISION II.

THE PHYSIOLOGICAL PROCESSES OF PLANTS.

THE physiological processes of plants are many, diverse, and complexly interconnected. Yet for purposes of study they must be separated and classified, and a convenient grouping, partly natural and partly artificial, is the following:

Section 1. The processes of NUTRITION, including all those concerned with maintenance of the life of the individual, involving the absorption, transformation, and release of matter and of energy.

Section 2. The processes of INCREASE, including all those concerned with expansion both in size and number.

Section 3. The processes of ADJUSTMENT, including all those concerned with adjustment to the conditions under which the plant must live.

SECTION 1. THE PROCESSES OF NUTRITION.

The processes of Nutrition are those which concern the maintenance of the life of the individual, and therefore they deal with the absorption, transformation, and release of matter and of energy. They fall, for convenience of study, into two somewhat natural sub-sections, according as they deal mainly with chemical changes or with physical movements, and they may be further classified as follows:

Sub-section A. Metabolic (chemical) processes, which include changes of:

a. CONSTRUCTION (synthesis or anabolism) of simple and stable into complex and less stable substances, with change of kinetic to potential energy; occurs in plants alone. Of the substances constructed there are two basal types, thus determining:

I. Carbohydrate construction, effected through:

1. *Photosynthesis*, utilizing energy of light.
2. *Chemosynthesis*, utilizing energy of chemical affinity.

II. Proteid construction, or

3. *Synthesis of Proteids*, utilizing energy of uncertain origin.

b. TRANSFORMATION (metabolism proper, assimilation proper) of the afore-mentioned synthates into related substances of special function, with only slight chemical, though often marked physical, changes, and without great change of energy relations; occurs in both plants and animals. Includes but one process:

4. *Conversion*, including *Accumulation* of reserves, and *Secretion* of substances of special function.

c. DESTRUCTION (analysis, reduction, katabolism) of complex and less stable to simpler and stable substances, with change of potential to kinetic energy; occurs in both plants and animals. Includes but one distinct process:

5. *Respiration* (*Energesis*), inclusive of *Fermentation*.

Sub-section B. Translocatory (physical) processes, which include movements of:

6. *Absorption* of materials into the plant.
7. *Transport* of materials through the plant, including especially:
   (*a*) *Transfer* of water, and (*b*) *Translocation* of plastic substances.
8. *Elimination* of substances from the plant, including *Transpiration* and *Guttation* of water, and *Excretion* of waste materials.

1. PHOTOSYNTHESIS.

Observation of the plant kingdom shows that plants fall into two primary physiological classes. The first includes the familiar green plants. These form the great bulk of vegetation, and embrace those kinds which best exhibit the typical and characteristic plant form, structure, and habit; they all subsist upon materials which they take from the earth and the air. The second class includes the parasites and saprophytes. These are not green, are insignificant in bulk, are dependent for their subsistence upon other organisms either living or dead, and are mostly the degenerate descendants of green plants. It is evident, therefore, that the green color, or *Chlorophyl,* is correlated with the dominant, the typical, even the ideal state of plant life, while its possession constitutes the most distinctive feature of plants. From these considerations it is obvious that Chlorophyl must have some meaning of fundamental importance in the physiology of plants, and this meaning the student must now proceed to determine. Logically the first problem presented is the following:

What are the essential facts about Chlorophyl, especially its exact mode of occurrence, its physical and chemical nature, and its properties?

This may be determined *first* (unless the matter be already familiar to the student from his earlier studies) by exact observation, necessarily microscopic, of its occurrence in favorable living tissues, and *second* by a study of its various optical and other qualities, which may most conveniently be made upon a solution of the substance in alcohol.

Observation. Select a very thin living green leaf, such as that of a moss, fern prothallus, or *Elodea;* mount it in water for the microscope, and note the appearance, location, and other visible features of the chlorophyl, expressing the results of observation in colored and annotated drawings. Later extend your knowledge of this subject by study in the best accessible books.

Experiment. Select the best available of the materials mentioned below; place about 100 sq. cm. of the leaf in a loosely stoppered test-tube or conical flask, with about 60 cc. of alcohol, warm in a water-bath kept at 50°–55° until the leaves are blanched (which requires but a few minutes); pour the solution into a clean test-tube, and (*a*) examine its color, both by transmitted and reflected light; (*b*) examine its fluorescence, which may be greatly heightened by focusing light into its interior with a lens; (*c*) examine the effect of its continued exposure to bright light, for which purpose it is better to divide a fresh solution into two equal parts kept side by side, one in light and the other in darkness; (*d*) describe these phenomena clearly, with such explanation of them as you can gather.

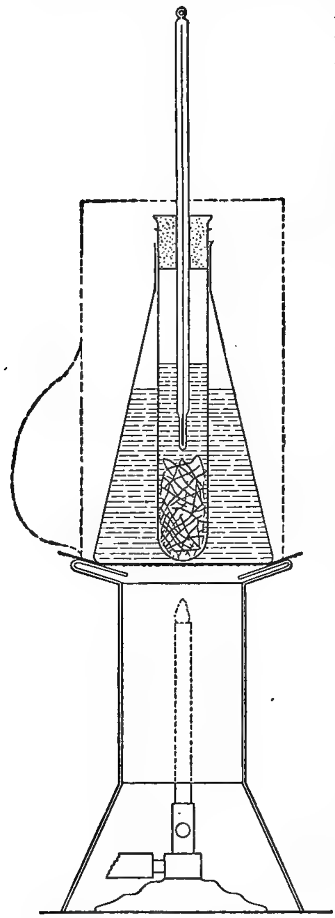


FIG. 14.—EFFICIENT ARRANGEMENT FOR PREPARATION OF ALCOHOLIC SOLUTIONS OF CHLOROPHYL; $\times \frac{1}{3}$.

The test-tube stands in a conical flask covered by a tin hood.

PRECAUTIONS. Care must be taken that the flame does not reach the inflammable alcohol or its vapor, to guard against which it is better not to attempt to heat the test-tube without the water-bath. A good water-bath may be extemporized from a beaker of water placed on an asbestos wire support over a Bunsen or spirit flame. A very efficient arrangement is shown by the accompanying figure 14. *Or*, if there is no haste, the dish may simply be stood on a radiator or other warm place, when the color will come out in an hour or two. Some leaves (not those mentioned below) do not release the color readily unless they are first boiled in water for a minute or two, but it is much better to omit the boiling, which causes chemical changes, whenever possible. Since the chlorophyl decomposes in light, it must be extracted under a cover, or at least not in bright light. The solution can be made stronger by use of more leaf, though it is apt not to be clear unless filtered. Solutions thus obtained are as good for study as those made by the very elaborate method of DETMER, 21.

MATERIALS. The best materials for this study are given in MISS ECKERSON'S paper upon chlorophyl spectra, cited on page 82. Thin, clear-green, half-mature leaves are in general the best; especially good are String Bean, *Abutilon*, *Primula obconica*, Squash, Grasses, Garden Nasturtium. Some common leaves, however, especially those of a yellowish-green color, such as *Pelargonium* species and *Oxalis Bowiei*, yield a yellow-green and less typical solution. For fluorescence the best materials, in order of excellence, are *Jacobinia magnifica* (very striking), *Cineraria*, *Cestrum elegans*, and English Ivy.

ALCOHOLS. All three of the common laboratory alcohols are good for this purpose. They are (1) the most-used ethyl alcohol or "spirits of wine," C_2H_6O, boiling at 78° (costing about 90 cents a quart retail, but about 10 cents to institutions with tax-free privilege); (2) methyl or "wood" alcohol, CH_4O, boiling at 66° (costing about 35 cents a quart); and (3) denatured alcohol, called also methylated or Columbian spirits, a mixture of the two preceding (costing about 30 cents a quart). The ethyl alcohol is sold in a 95% strength; the strength is determined by a special specific-gravity alcoholometer. In making up various strengths with water, the proper quantities of both must be measured out separately and then poured together, since there is a shrinkage when they are mixed, resulting in erroneous volumes if one is added to the other in the same measuring-glass. Alcohols are best kept in glass-stoppered bottles. Upon the iodoform test for alcohol, see under Fermentation later.

DEMONSTRATION. That chlorophyl can be removed from green tissues, leaving them white, may easily and strikingly be shown to an entire class as follows. Prepare an arrangement like that of Fig. 14, but of the largest practicable size and without the hood, which is needless away from direct sunlight. Bring the water to boiling and put out the flame; take from String Bean, *Tropæolum*, or *Abutilon*, an adolescent leaf with a long petiole and with a blade less than half the length of the test-tube; by petiole (and forceps if necessary) dip the leaf for a minute in the hot water, and then drop it, loosely rolled, (not crumpled), into the test-tube; fill the latter two-thirds full of alcohol, and insert a grooved stopper, which is to press against the petiole so that this will hold the blade towards the bottom of the test-tube. Lower the test-tube into the hot water, when the alcohol will soon boil, and the color will come out rapidly and show very clearly, especially against a white background. After it has thus boiled for five to ten minutes, pour off the alcohol and replace it by water, when the leaf will show white. No doubt, if it were worth while, the process could be projected upon a screen.

The chemical and physical constitution of chlorophyl must now be considered by the student. Although some of the simpler phases admit of ready experimental study, the more fundamental matters do not, and he may best work out the subject through the literature. Accordingly he should become acquainted with *(a) the composition, the conditions of formation (especially as to light and temperature), and the stability of chlorophyl in the living leaf; (b) the nature and supposed meaning of the associated colors, xanthophyl, erythrophyl, etiolin, carotin; (c) variegation in leaves; and (d) autumn coloration.*

One of the optical properties of a substance of the first importance is its power of differential absorption of light. This is investigated by means of the spectroscope, the principle of which the student must now thoroughly understand. It happens, as will later fully appear, that this property in chlorophyl is of fundamental physiological importance, which necessitates here this inquiry:

What is the absorption spectrum presented by chlorophyl?

To determine this it is simply necessary to examine spectroscopically the light passed through chlorophyl, either in the living leaf, or, more conveniently, in solution, and to compare this with the spectrum of unaltered light.

EXPERIMENT. Prepare a solution of chlorophyl freshly in the dark by the method of the preceding section, using the most available of the materials listed below. Place it in three vials, preferably flat-sided (or in small Soyka flasks); support these in front of the slit of the spectroscope, which is directed towards a convenient white light, such as incandescent, electric, or Welsbach; examine the spectrum in various thicknesses in comparison with an unaltered spectrum formed by the comparison prism. Make sure, by way of control, whether any of the observed effect is due to the solvent. Chart the chlorophyl and the pure spectrum in colors, using a scale to ensure correct proportions and position, and add the usual notation.

MATERIALS. These are fully discussed, from the present point of view, with an account of methods and precautions, by MISS ECKERSON in the Botanical Gazette, 40, 1905, 302. As there recorded, the most typical spectra, in order of excellence, are yielded by *Primula obconica*, Radish, Horse Bean, *Abutilon* young leaf, seedling Oats, *Cestrum elegans* young leaf, Poinsettia young leaf, Tomato, Chinese Primrose, and Castor Bean. A more complex spectrum, with additional bands indicative of incipient decomposition, is shown by acid leaves of a yellow-green color, such as *Pelargonium* species, *Begonia coccinea, Oxalis Bowiei.*

SPECTROSCOPES. The absorption bands in the chlorophyl spectrum are so prominent that any spectroscope, however simple, shows them clearly. Very convenient for their examination is the KIRCHOFF and BUNSEN student form, costing about $30.00. It is greatly improved for the purpose if there is added a proper support for the vials, together with an adjustable mirror for lighting the comparison prism, and another for the scale, thus permitting the entire instrument to be used with one electric or other light. The complete arrangement is shown diagrammatically by Fig. 15. Much more compact, and nearly as efficient, though lacking the advantage that the principle can be readily shown, is the direct-vision spectroscope. One of these, especially prepared for student use, is among my normal apparatus, and is figured herewith (Fig. 16). It is a Browning instrument with comparison prism, the whole clamped in a frame which carries in front of the slit two spring clips arranged to hold a small flask, or vial, of chlorophyl solution, which of course can be made of greater or lesser density. It may be set in front of a light, when the bands may be studied and mapped at leisure, or, the base being unscrewed from the handle, it may be passed around the class for demonstration, each student directing it towards some selected source of light. A microspectroscope, for use with the microscope, as described by DETMER, 23, may also be used, but is very expensive, and for our present purpose has no advantage over the above instruments. Different thicknesses of solution may most effectively be secured by use of triangular troughs sold

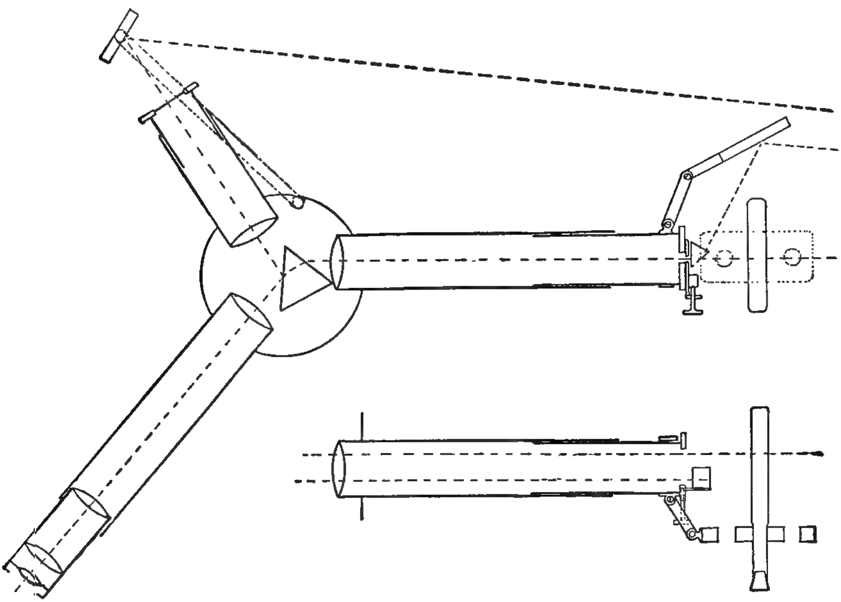


FIG. 15.—AN EFFICIENT ARRANGEMENT OF THE SPECTROSCOPE FOR THE STUDY OF CHLOROPHYL; UPPER SECTION AS SEEN FROM ABOVE, LOWER FROM THE SIDE, $\times\frac{1}{4}$.

The dotted lines show the path of light, reflected in part by mirrors, from a single incandescent lamp. The chlorophyl is in the flat (Soyka) flask.

by dealers in chemicals, though the cement of some of these is attacked by alcohol. Very good and well-colored pictures of chlorophyl spectra are in the FRANK and TSCHIRCH diagrams (page 23), Plates XV, XVI.

DEMONSTRATION. The chlorophyl spectrum may be shown to a class, though not to all simultaneously, by the hand instrument above described and figured (Fig. 16). It should be possible to project the spectrum finely upon a screen with a projecting spectroscope.

The student may also care to examine the spectrum of the living leaf. This requires some special manipulation, as do some other matters of correlated interest, which may be experimentally studied by aid of the following suggestions:

SUGGESTED EXPERIMENTS. *The Spectrum of Chlorophyl in the Leaf.* Ordinarily this does not show well if the living leaf is held before the spectro-

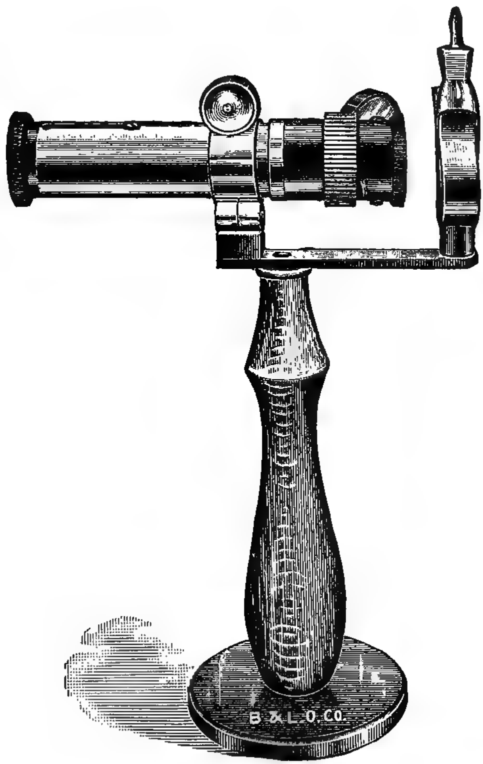


FIG. 16.—SPECTROSCOPE, STUDENT FORM; ×⅜.

scope, because the air in the intercellular spaces refracts and scatters the rays; but if the air be replaced by water (by immersing the leaf and exhausting the air above it), the leaf becomes translucent, and its spectrum is plainer, though the examination must be made at once, for the chlorophyl alters quickly; *or* the leaf may be simply boiled in water, though this is less effective, since it produces alterations in the bands. The leaf should be examined in water, either in a vial or between glass plates.

The Light from Dense Solutions. The color of the transmitted light just before it is finally extinguished by increasing thickness of solution of chlorophyl is of great interest in connection with its spectrum. It may be observed by use of a very dense solution, made up from dark-green leaves placed either in a row of vials from which side light is cut off, or in a wide crystallizing dish. It should also be viewed without the spectroscope, for which purpose it may be placed in a long test-tube wrapped with black paper and held over a light-reflecting mirror. The same phenomenon can be observed in living leaves by use of the diaphanoscope of SACHS (Gesammelte Abhandlungen, 1, 169), a simple instrument readily adapted from concentric cardboard cylinders blackened inside and arranged to hold discs of leaf, as shown by the accompanying figure (Fig. 17). The eye is placed at the open end, with the discs directed towards a bright light. The longer cylinder should be about the length of clear vision, *viz.*, 25–30 cm.

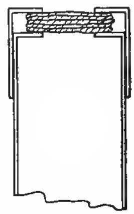


FIG. 17.—DIAPHANOSCOPE, WITH LEAF DISCS IN POSITION, IN LONGITUDINAL SECTION; $\times\frac{1}{2}$.

Colored Foliage Leaves. Many of these, such as *Coleus*, etc., seemingly red, may be shown to possess chlorophyl by placing them in hot water or alcohol; and this is true, also, of some brown and red seaweeds.

The student will of course make sure by review, or otherwise, of his knowledge of the physical significance of the black bands in relation to light energy. The importance of the subject will be evident later.

Turning for a time from the properties of chlorophyl to its distribution, if the student will observe its places of occurrence through those plants which possess it, he will see that it occurs only in the parts exposed to light, and moreover is most abundant where the light is greatest. This implies some very close connection between the use or other meaning of chlorophyl and the presence of light. To test the existence of such a connection, the natural first step is to observe the effect of withholding the light from green tissues, and thus is presented the following problem:

What differences develop between similar green tissues kept for some time respectively in and away from the light?

To determine this it is only necessary to screen a green tissue from light, leaving a control portion exposed; but the screening must be done in such a way that the screened and unscreened portions are under precisely the same conditions except as to light, are not seriously disturbed in the performance of their usual functions, and are as closely alike to start with as possible, for which latter reason they should be parts of the same plant, or, better yet, parts of the same leaf.

EXPERIMENT. Select a nearly grown leaf from the best of the materials mentioned below, and, in the early morning, place on it a normal light screen; leave the plant in bright, but not intense, light for a full day, and towards evening examine it for any observable macroscopic or microscopic differences between the lighted and the darkened portions. Then blanch it by removing the chlorophyl as described earlier (page 79), and examine again. Then apply the first of all microchemical tests,—a solution of iodine,—and explain the visible result.

PRECAUTIONS. In order that the screen may be attached early to the plant, it may be put in place the evening before. In practice, however, much more certain results are obtained with most plants if they are first placed for a day (or two if it is cool) in a dark chamber, in which case the screen need remain attached to the plant only an hour, or even less, after it is brought into the light. The plant should never be stood in direct sunlight, which might burn the screened leaf, and the temperature should not be allowed to rise above about 22° (about 20° is best) for reasons which will appear later under Translocation. Also with most leaves the blanching is quicker and the result is plainer if the leaf is placed in boiling water for a minute or two when removed from the plant. If the leaf is to be handled, the alcohol should be replaced by water for a minute or two to remove the brittleness the alcohol causes. The leaf blanches much better if flat or loosely rolled, since crumpling or folding prevents free circulation of the alcohol. In using the test with some thick leaves it will be found that they yield the chlorophyl slowly; the blanching may be hastened by addition of a little caustic potash to the water. Sometimes, also, because of the presence of brown coloring-matters, they show the iodine-blue but badly. Hence it is best to use thin soft leaves.

MATERIALS. These have been investigated from this point of view in my laboratory by MISS ECKERSON, whose paper upon the subject is expected to appear later, probably in the Botanical Gazette. She finds that the best leaves for yielding a marked and rapid response in this experiment are, in approximate order of excellence, Sunflower, Fuchsia, String Bean, Horseshoe Geranium, Squash, Castor Pean, Indian Corn, and seedlings of most common plants. All in this list, if previously kept for a day or two in darkness, will show some starch in from 15 to 20 minutes, and an abundance of starch within an hour. The Fuchsia, Sunflower, Castor Bean, and most seedlings ordinarily need not be kept in darkness longer than one night and the morning up to the time of beginning the experiment.

Compare also the important list given by MEYER in Botanische Zeitung, **34,** 1885, 417 (synopsis in JOST, 111).

NORMAL LIGHT SCREENS. It is quite wrong, in good experimenting, to use as light screens any discs of cork, tin-foil, black paper, etc., applied either to one or to both sides of a leaf, partly because the lighted and unlighted upper surfaces are not otherwise under the same conditions, but especially because there is serious interference with the normal leaf-functions. Screens correct in principle must allow for free access and exit of gases, but in their construction advantage may be taken of the fact that in ordinary leaves the stomata are either largely or wholly on the under surface, so that if this is left free the upper surface may be covered as closely as desired. It is upon this principle that I have constructed the two forms (described in the Botanical Gazette, 43, 1907, 277) which are supplied among my normal apparatus (page 46), and which, I believe, offer a logical and very efficient and convenient method of screening the leaf. The larger (Fig. 18), designed to take

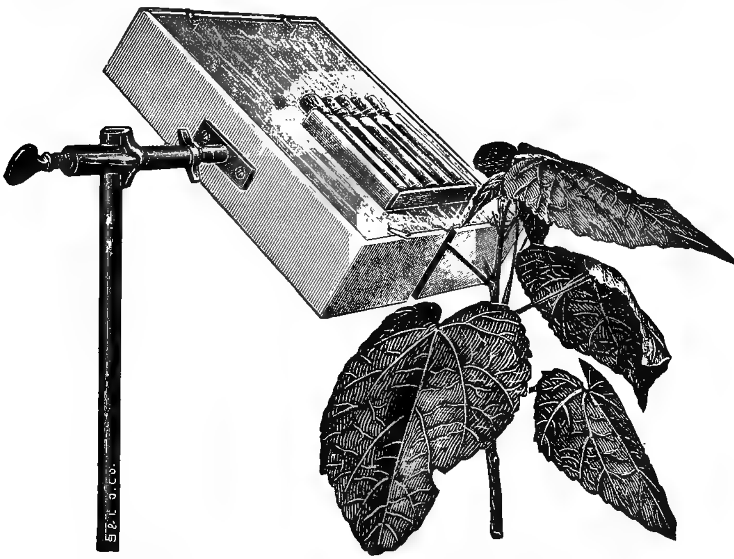


FIG. 18.—NORMAL LIGHT SCREEN ADAPTED TO SEVERAL USES; $\times\frac{1}{4}$.

an entire leaf of moderate size, consists of a wooden box readily adjustable for height and angle, $5 \times 7 \times 1\frac{3}{4}$ inches (internal), white without and black within, separated lengthwise into two compartments, with an intermediate space for petiole and midrib. The bottoms of the compartments are largely open, and so matched by diaphragms that air can enter freely, but direct light cannot. Movable gratings of silk threads hold the leaf firmly but elastically against the glass cover, which may be either two separate strips covering the compartments (and therefore the halves of the leaf) or a single sheet 5×7 inches in size. The cover may carry tin-foil, cut with any desired pattern, gummed to its under surface, thus forming a screen in which the light and dark parts are under conditions otherwise practically alike; or it may carry vials of pure colors correlative to the light and dark parts of the chlorophyl spectrum, as was the case when the accompanying photograph was taken, the meaning of which will be later explained; or it may be replaced by a 5×7 negative for GARDINER's striking starch-printing experiment (later mentioned). The arrangement does not, of course, permit as free access of carbon dioxide as the uncovered leaf enjoys, but this is only a matter of degree and does not affect the result when sufficient time is allowed for the experiment.

The smaller screen (Fig. 19), of a lesser range of general usefulness, though ample for demonstration of the present subject, is made upon the same principle, except that it is arranged to clasp a portion of a leaf. A spring clip holds a glass disc against the upper surface of the leaf, which is

FIG. 19.—SIMPLE NORMAL LIGHT SCREEN; ×½.

supported below by a grating of threads stretched across the top of a ventilated dark box. The glass is removable from the clip and may carry a tinfoil screen cut with a pattern and gummed to its under surface as in the figure, or it may be used to hold a photographic negative (film or glass) against the leaf.

Simpler forms, embodying the same principles, may be adapted from common materials, and even make-shift forms are possible. For demonstration purposes it is not essential that the under side of the leaf shall be darkened, especially if the background is dark, but, for the most part, the results are better when this is done. It is possible to adapt a fair normal screen from the leaf-clasp described later under Transpiration, by placing the pattern upon a glass held by one ring against the leaf, and attaching a ventilated paper box to the opposite ring.

DEMONSTRATION. This important experiment can be shown to an entire class by following upon a large scale the general methods given in this and the preceding sections. The large-leaved plant, after a day or two in darkness, followed by two or three hours' exposure to bright light with normal screen attached, is brought on the table beside the blanching apparatus (of Fig. 14). The leaf is removed, immersed in the hot water, kept under alcohol in the test-tube until white, covered with water for a minute or two, placed in a flat glass vessel standing on a white dish or tile, and covered with iodine solution; then the pattern should soon appear. It may be passed thus around the class, or the leaf may be removed, placed on the white tile, and held up for inspection. A very striking result is given by use of a photographic negative as a screen. It is possible to preserve the leaves permanently in 50% alcohol, and although the pattern fades out, it can be restored at any time by fresh application of iodine.

Dark Chambers. When plants are to be kept for a day or more in darkness, the conditions should be in every respect as nearly normal as possible. Best of all for this purpose, and most convenient, is a ventilated dark room, built into and taking the temperature and moisture of the greenhouse itself, as described earlier in this book (page 34). Next in value is a ventilated dark box, upon a similar principle (page 43). After this in efficiency would come the normal small chamber later described under Growth; it is readily adaptable to this purpose. Improvised arrangements should ensure healthful conditions of temperature, moisture, and purity of air.

Iodine Tests for Starch. One of the most valuable and striking of all tests for organic substances in the plant is the blue color imparted to starch by contact with iodine in solution, due to the formation of the blue iodide of starch. The test has the advantage that it may be used either upon a microscopical or a macroscopical scale, its striking advantages for demonstration having first been shown by Sachs in 1884 (Arbeiten des botanischen Instituts in Würzburg, 3, 1884, 1, and his Gesammelte Abhandlungen, 1, 1892, 354). Iodine (I) is a flaky-crystalline, black-violet lustrous solid, brown in solution, very sparingly soluble in water (about 1 g. in 5200 cc.), but dissolving readily in water containing alcohol or potassium iodide (KI). The latter is the most useful and inexpensive solution for use on a large scale; a good strength is,—iodine 1 gram, potassium iodide 5 grams, water 1 liter (most quickly made by placing the potassium iodide in about 50 cc. of water, adding the iodine, and, when all is dissolved, pouring the solution into the remaining water). The tincture of iodine of the druggists, diluted with about 20 times its bulk of water, is also good. These strengths are much weaker than commonly recommended, but, in my experience, are amply strong. Very much stronger solutions are, however, advantageous for microscopical use, and Strasburger recommends iodine 5 cg., potassium iodide 20 cg., water 15 cc. Iodine, as well solid as in solutions, and even in some combinations, is readily volatile, and hence can only be preserved in closely stoppered bottles.

At times iodine does not immediately stain the starch of living cells, because of an enveloping film of living protoplasm. Immersion in hot water, however, will kill the protoplasm, swell the grains, and show the reaction at once.

Erroneous Experiment. One of the most familiar experiments of the text-books is that in which two corks are pinned matching on the two faces of the leaf to serve as a light screen, the resultant white circle in the iodine-treated leaf being attributed to absence of light. As a matter of fact this inference is fallacious, as any control experiment would show, for the result is almost equally due to absence of the necessary carbon dioxide. This has been shown by MISS HAUG in the Botanical Gazette, **36**, 1903, 389, and by C. A. KING in Torreya, 5, 1905, 67, and the Plant World, **8**, 1905, 263. The corks do not entirely exclude this necessary gas, for some can leak between cork and leaf, possibly a little through the cork, and a trifle from neighboring intercellular spaces; but they exclude it from all the central part; and the leaf remains just as white, except for a shaded margin, if the cork be replaced by a suitable control arrangement of cork and glass. The experiment was used originally by SACHS to show translocation of starch from unlighted areas, and here it is quite correct, but since his time it has been erroneously applied by many.

The student is here in contact with one of the most significant facts in all organic nature, *viz.*, the appearance of starch in lighted green leaves. He may care, therefore, to experiment with it somewhat further, in which case he will be aided by the following suggestions:

SUGGESTED EXPERIMENTS. *Microscopic Observation of the Action of Iodine on Starch.* Cut sections of certain leaves, then blanch and clear them either by strong chloral hydrate, or by strong caustic potash (afterwards washed out); mount the sections in water, observing them with the microscope, and apply iodine solution. Directions for accomplishing the whole process in the neatest manner are given by DETMER, 46.

Relation between Intensity of Light and Amount of Starch. This may be well shown by using a screen composed of narrow strips of thin translucent paper, arranged in thicknesses of one, two, three, etc., thus varying the intensity of the light without altering its quality.

But it may be shown far more strikingly by the method recommended by GARDINER (Annals of Botany, 4, 1889, 163), in which the screen is formed by a photographic negative. In this way a positive photograph may be printed in starch on the leaf, and "developed" by iodine. It is essential that the negative be held pressed flat against the leaf, which is well and conveniently accomplished by the normal light screens earlier described (page 87); and it is also better to select a naturally flat leaf, such as *Abutilon* or *Phaseolus vulgaris* (String Bean).

Time Required for the Appearance of Starch. This may be determined effectively as follows: Place a plant for one or two days in the dark (when it empties itself of starch by translocation, as will later be noted); bring it into bright light, keeping it at a temperature of 20°–22°; take from it at regular five-minute intervals small discs of tissue, and mark them; later blanch these and apply to them the iodine test. The leaf-area cutter, later described, is very convenient for this, but a cork-borer working against a cork is also good.

Disappearance of Starch from Green Tissues in Darkness. This may be demonstrated either by SACHS' method, described in DARWIN and ACTON, 32, or by the reverse of the preceding experiment, *viz.*, taking a suitable plant from the bright light about ten o'clock of a bright day (by which time it usually contains an abundance of starch), placing it in a dark warm (25°–30°) place, and taking discs from the leaves at regular intervals, these being marked and later tested for starch. This Translocation of starch will be considered later under that subject.

In connection with the foregoing experiments the student has been brought into touch with questions concerning the optimum temperature and light for promoting the process under study. Our knowledge of this matter is now in a transitional state, and the subject is set forth with great clearness, under the name Assimilation, by BLACKMAN in the Annals of Botany, **19**, 1905, 281. This paper the student should here consult, noting particularly his temperature-curve and its meaning.

PRACTICAL OPTIMA OF TEMPERATURE AND LIGHT IN PHOTOSYNTHESIS. As BLACKMAN shows, in the paper just cited, the rate of photosynthesis probably rises with increase of temperature to near the death point, the optimum and maximum thus lying near together. Since, however, at temperatures above 25°–30° the rate is not maintained when the temperature is held constant, but falls off (and the faster, the higher the temperature), there is no experimental advantage in working with temperatures above about 25°, the more especially as respiration increases so rapidly with higher temperatures as soon to quite equal and then overbalance photosynthesis. Further, if one is working with starch formation in the leaf, it is in any case necessary to keep it below 25°, since above that temperature, with most leaves at least, the translocation is so fast that no starch appears in the leaf even though an abundance of the photosynthate is being formed. All things considered, for experimental purposes the optimum may best be taken as about 22°, certainly not above 25°.

As to the optimum of light for photosynthesis, BLACKMAN has shown that this depends upon the percentage of carbon dioxide available to the leaf. Where, however, the percentage is as low as it is in the atmosphere, the leaf cannot use, especially in summer, all the light of direct sunlight. A strong diffused light, therefore, not only contains all the light the leaf can use, but it is better for the leaf in other ways, particularly if the tissues are enclosed.

The foregoing observations upon the appearance and disappearance of starch must suggest to the thoughtful student the query, whether this substance represents simply a transformation of some leaf material already present, or a new substance added to the leaf. This is an important subject which presents a definite problem as follows:

Is the formation of starch in leaves accompanied by an increase of substance?

This may be determined by a comparison of dry weights (dry to exclude the effects of varying quantities of water), of identical green tissues before and after they have made starch.

Experiment. Select a large plant with somewhat thin leaves of rather firm texture, such as one of the three common Geraniums, or Fuchsia, and keep it for a day in the dark, or two days if low temperatures prevail. On a bright morning bring it into a good light, and, by aid of a leaf-area cutter, cut as large a number of discs as possible, preferably at least 100, from the leaves, taking them alternately from the soft parenchyma of the right and left halves, but leaving ample tissue for an equal number to be cut from the same leaves later. Suspend the discs for two or three minutes over boiling water in a test-tube, in order that the steam may kill them and prevent loss by respiration; then place them in a drying-oven. Two or three hours later cut 100 discs from the same leaves, kill them, and place them in the oven. Dry both sets, giving them at first a temperature of about 60°, raised gradually to near 100°, until they cease to lose weight, which will require about two days. Weigh both sets to milligrams, compare the results, and reduce to grams per square meter per hour.

Precautions. The sets of discs should be cut alternately from the two sides of the leaves, and not, as usually recommended, from one side or the other, since, as Miss Eckerson has discovered, the leaves of any given plant commonly have one side or the other prevailingly heavier. It is best to take the largest possible number of discs, since the error from local variations in thickness, etc., tends thus to disappear; but at the same time too large a number should not be taken from the leaves lest it do them injury. It is, however, a fact, readily shown by experiment, that the removal of the discs does not seriously disturb the functioning of the leaves. Two, or at most three, hours of exposure to light are better than a longer time, since beyond the former period the accumulation usually becomes much slower, thus reducing the average increase per hour. Brown and Escombe, in a paper of 1905, cited below under Literature, claim that this method gives results much too high, but of this I am not convinced.

Leaf-area Cutters. These were introduced in principle by Sachs, when he devised this method (Arbeiten des botanischen Instituts in Würzburg, 3, 1884, 19, and Gesammelte Abhandlungen, 1, 372), but his way of cutting areas with a knife by aid of a pattern was cumbersome. Using this general method, however, I have developed an instrument permitting facile and accurate work in this experiment. It is among my normal apparatus (page 46), and, as improved considerably from the original form described in the Botanical Gazette, **39**, 1905, 150, is constructed as follows.

The cutter works on the principle of a punch (Fig. 20). A stiff iron frame, of a form conveniently grasped by one hand, carries steel dies operated by pressure of the thumb. The dies cut discs cleanly from a leaf held between them, the discs then dropping into a perforated aluminum cup screwed below the lower die. The diameter of the punch-dies is as nearly as possible 1.128 cm., and hence every disc is 1 sq. cm. in area. In use the arms of the frame are slipped above and below the leaf, which is guided by the left hand, when the discs may be cut very conveniently and rapidly, in any desired number, care being taken to avoid the larger veins. The cup is then unscrewed and covered by its own screw cap, which projects sufficiently to allow the cup to

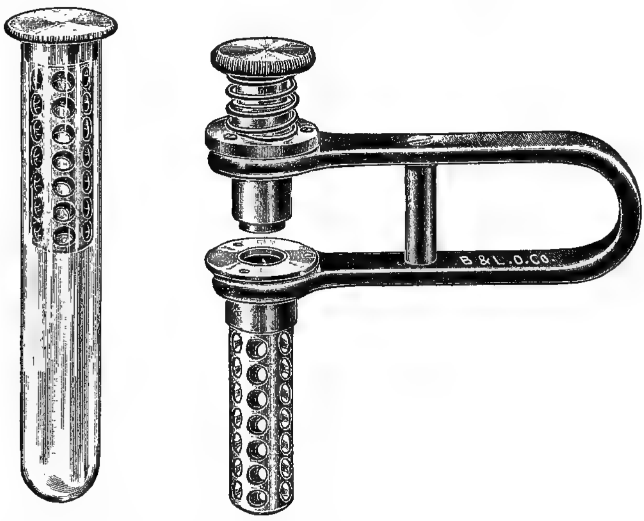


FIG. 20.—LEAF-AREA CUTTER, WITH DISC CUPS; $\times\frac{3}{8}$. ON THE LEFT IS A CUP IN A TEST-TUBE AS ARRANGED FOR STEAMING THE DISCS.

hang near the top of an ordinary test-tube, as shown in the figure. Water in the bottom of the test-tube is then boiled in the gas-flame (any convenient holder being used), and the steam enters the perforations of the cup and kills the living cells of the discs, thus preventing any loss of weight by respiration. The cup, with its contents, is then placed in the drying-oven. After a predetermined interval, using the other cup, an equal number of discs is cut from the same leaves. These are treated as in the first series, and the cup is placed beside the first in the drying-oven. When the discs are thoroughly dry, which requires two or three days, both cups are carefully weighed; then the weights of the cups (stamped upon them, together with the letters *M* and *N* respectively to distinguish morning and night cups) are subtracted, when the remainder gives the dry weights. Both cutter and cups should often be cleaned, to prevent rusting of the one and accumulation of dry sap on the other. The cups should of course be weighed when new to make sure of the correctness of the stamped weights; and they should occasionally be weighed thereafter. Some thin leaves show a tendency to stick to the upper die, and hence the discs do not enter the cup. This difficulty can be overcome by painting the face of the upper die with a thin glaze of water-glass.

An area cutter of fair efficiency may be adapted from a cylindrical cork-borer working through the leaf against a cork, the discs remaining in the tube; they may be supported in the test-tube by a wire diaphragm, and weighed vials may then serve in place of the aluminum cups.

Demonstration Methods. The increase of dry weight in leaves in presence of light may readily be shown to a class by this method, though naturally it cannot be made very striking.

Other Methods of Study. Another method, quite different in principle, of determining the amount of photosynthate formed in the leaf has been used by Brown and by Blackman, as described in their papers cited below under Literature. They enclose the leaves in special chambers, supply carbon dioxide in known amounts, and determine the quantity used by the leaves in a given time, from which, of course, the amount of photosynthate can be calculated. The results are much lower than those obtained by Sachs by the leaf-area method just described, but the subject is not yet closed. There is also an apparatus of similar aim described by Stone in Torreya, 4, 1904, 1.

It will occur to the student as he completes the preceding experiment, especially if he has reduced his results to grams per square meter per hour (gm^2h), that this result represents the actual amount of starch (or equivalent) made in that time; and he will think thus to obtain one of those exact quantities which it is the aim and the delight of the physiologist to secure. Unfortunately, however, the result is seriously affected by a loss due to the disappearance of some starch through translocation and respiration, processes later to be considered. This loss can in part be prevented, however, and in part computed; and if the student can follow farther this very important, though rather difficult line of study, he will be aided by the following:

Suggested Experiment. Following the general method of the preceding experiment, prevent the translocation loss by the method which Sachs describes (in his paper last cited, 372; also Detmer, 49), and compute the respiration loss from the Table of Conventional Constants in Part III of this book. The result should be compared with the corresponding figures in the same Table of Constants. Compare also the references in Jost, 116.

Brown and Escombe, in a paper of 1905 cited below under Literature, 52, dispute SACHS' interpretation of the increased weight in this experiment, holding it is due to a more free admission of carbon dioxide through more widely open stomata.

Viewing the foregoing studies rather broadly, it may seem to the student that while the formation of starch has been shown to be accompanied by an increase in weight in the leaves, this increase may simply represent material drawn from the stem, and not new material added to the plant. If practicable it will be of interest to test this possibility, which may be done by including entire plants in the experiment, instead of leaves only, though it may also be accomplished by the use of leaves severed from the plant.

SUGGESTED EXPERIMENTS. To obtain comparative weights of organic substance in different entire plants, it is obviously necessary to eliminate the soil, which can be done by growing the plants through water-culture methods, which are described by DETMER, 1, and others, and later in this book (under Synthesis of Proteids). For the present purpose, however, it may very readily and satisfactorily be accomplished as follows. Take three sets, each of 100 seeds, of Japanese Buckwheat, Radish, or Oats, and weigh each set to a milligram, preferably, though not necessarily, making the three sets equal in weight. Keep one set in reserve, and spread each of the others, after suitable soaking, in a five-inch flower-pot saucer thoroughly cleaned by boiling. Set these saucers in crystallizing dishes of such a size that the saucers rest on their projecting rims (Fig. 21). Keep the dishes supplied with tap water, which will supply itself in correct quantity, and filtered, to the plants. *Or* a hydrostatic arrangement may be used for the watering. Expose the two sets of seeds to similar and good growth conditions, preferably under similar bell jars, except that one set is screened from light. Keep them under constant care until those in the dark have reached their limit of growth, which will require three to five weeks according to season. Remove the plants, with all seed-coats, etc., from the saucers, to which they will not adhere, place them in suitable glass dishes, and dry them in the oven, together with the third set of seeds, until they cease to lose weight. Then weigh them all carefully, using the weight of the seeds to determine the percentage of water originally present in the seeds of the other sets, and determine the exact increase or decrease of dry weight. Two apparent errors in the result, a loss from

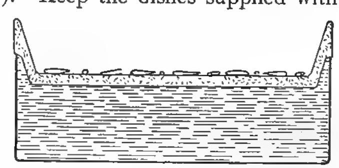


FIG. 21.—ARRANGEMENT FOR SPECIAL MODE OF WATER-CULTURE, IN SECTION; ×⅛.
Seeds rest on a flower-pot saucer kept wet by water in a crystallizing dish.

respiration and a gain from absorption of minerals, will be approximately the same in both.

The foregoing experiments will make it plain to the student that he is here concerned with a process of formation, or synthesis, of a substance under the action of light, a process appropriately known as *Photosynthesis*, while the substance made may appropriately be termed the *Photosynthate.*

If he experiments at all widely upon starch formation, the student cannot fail to observe that different plants give the starch test with very different degrees of readiness, some of them giving none at all. It becomes now a question of great interest whether the latter plants increase in weight, a matter which the student should determine by experiment if conditions allow, otherwise from the literature.

SUGGESTED EXPERIMENT. Using the general method of page 92, but applying it to some plant which shows no starch (an *Allium*, *Scilla*, or some other in the list by MEYER, Botanische Zeitung, 1885, synopsis in JOST, 111), ascertain whether there is any increase in weight of the green tissues in light.

This experiment brings the student face to face with other forms of the photosynthate, and also with the very important subject of its chemical composition. The subject involves problems in organic chemistry rather impracticable for experimental study in the present course, but the student must follow it through the literature. In this way he should carefully work out, and express in a clear exposition, *the present state of knowledge of the various forms of the photosynthate, their chemical composition, the stages in their formation, their interrelationships, and the reasons why starch appears in leaves.* He will be greatly aided in the study by some experimentation upon the simpler phases of the subject, on which he can find directions in DETMER, 46, and especially in DARWIN and ACTON, 276.

These studies will show that the chemical composition of the photosynthate approximates to $C_6H_{12}O_6$, which may be taken as a conventional formula for the photosynthate in general. This at once raises the question as to the source of supply of these elements. Scrutiny of the formula suggests that since the hydrogen and the oxygen are in the same proportions as in water, it is possible that a part of the material is derived from the water present in the leaves, and this is actually known to be true. Seeking a source of supply for the carbon, and noting the irregularity of its occurrence in the soil, the most obvious possibility is that it is derived from the carbon dioxide of the atmosphere, which presents the following experimental problem:

Do green plants use the carbon dioxide of the atmosphere in the formation of the photosynthate?

This may be tested most directly by analysis of the air in which plants carrying on photosynthesis have been confined. But such analysis, because of the smallness of amount of the carbon dioxide concerned, is difficult; and in practice the matter may much more easily be tested by the indirect method of placing equivalent green tissues in light under conditions precisely alike except that carbon dioxide of the air is allowed access to one, but is kept from the other, and noting whether any photosynthate, typified by starch, is formed.

EXPERIMENT. Take two two-neck Wolff bottles each of at least a liter capacity, and on each grind one neck flat on top, preferably making the ground tops the same height from the table. Cover the bottom of one bottle with a layer of a carbon-dioxide absorbent, preferably fresh soda-lime, and the bottom of the other with a layer of chalk, which is physically equivalent, but non-absorbing. Stopper the unground necks, and cover the ground surfaces with soft wax, described under Manipulation in Part III; bring the waxed necks close together, and press down upon them the two halves of a large leaf of a good starch-forming plant previously emptied of starch by a day or two of darkness, and attach the leaf firmly in place under glass slips clamped by wire springs, as shown in the figure (Fig. 22). After two or three

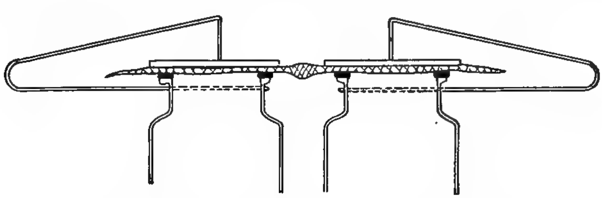


FIG. 22.—ARRANGEMENT FOR MAKING AN AIR-TIGHT CONNECTION OF A LEAF WITH NECKS OF BOTTLES.

Spring clips hold glass slips against the leaf which rests on soft wax against the necks of the bottles.

hours in bright light, remove the leaf, scrape off the wax, blanch the leaf, and apply the iodine test.

Carbon-dioxide Absorbents and Tests. These are frequently needed in experimentation, and before attempting to apply them to a physiological problem, the student should make himself familiar with their action by actual tests with known quantities of carbon dioxide.

(1) *Caustic potash* is the best agent for removing that gas from a confined space, absorbing it after the equation $2KOH+CO_2=K_2CO_3+H_2O$. It may be used in any strength from the weakest solution to the solid, the latter requiring some slight moisture for its operation. As a rule the absorption is the more rapid the stronger the solution, the higher the temperature, and the greater the agitation of the liquid. A useful solution is one of 5 g. commercial caustic potash to 15 cc. of water, this strength having the advantage that it is the same as that used later in connection with oxygen absorption (page 104). Of this solution 1 cc. will absorb about 100 cc. of carbon dioxide within three minutes (if agitated), but in practice it is best to allow only half that amount or less (Hempel recommends one-quarter). In making up the solution, advantage can be taken of the fact that the solid sticks in which commercial caustic potash is sold weigh, on the average, about 1 gram per centimeter of length (though the commercial sticks are only from 75–85% pure), and thus the sticks can be measured off accurately enough for practical purposes. A solution should always be made up at least fifteen or twenty minutes (preferably longer) before needed, in order to allow it time to cool, and also to lose the bubbles of air carried into the solution by the solid stick; and it is well to keep a stock solution in bottles tightly stoppered with rubber stoppers. It may most conveniently be applied by use of the reagent tubes later described (page 105). Since it is very diffusible and very disturbing to chemical processes into which it accidentally comes, all dishes which it has touched should be thoroughly washed; and since it is irritating to the fingers (and destructive to fabrics, etc.), it is well to handle the sticks with small tongs kept for the purpose. I have found that these ends may very advantageously be met by having a small wooden tray, about 25 cm. $\times$ 15 cm. $\times$ 3 cm. deep, with handles at the ends, in which the bottle containing the solid sticks (with a screw-eye in the cork for a handle) is kept wired in one corner, and to which the tongs and knife used in handling the substance are attached by light chains. In its bottom is a millimeter ruler by which the sticks may be measured, and thus roughly weighed. The tray holds also the stock bottles and any other dishes connected with the use of this substance.

(2) *Soda-lime* is a mixture of caustic soda and quicklime, obtainable in tight bottles; it forms an eager absorbent of carbon dioxide, very useful where conditions forbid the use of a liquid. It works somewhat better in lumps of appreciable size. It is much cleaner to use, though less efficient, than caustic potash.

(3) *Lime-water* is a saturated solution of calcium hydroxide in water, which absorbs carbon dioxide after the equation $Ca(OH)_2+CO_2=CaCO_3+H_2O$, the calcium carbonate appearing as a white precipitate. The appearance of the precipitate, thus making the absorption of carbon dioxide visible, gives this reagent a great advantage over the caustic potash as a test,

but, owing to the low solubility of the calcium hydroxide in water (only about .2%), the solution is too weak to form a rapid absorbent of the gas. It can be kept in tightly stoppered bottles, having an excess of lime on the bottom, from which it may with caution be poured off as a clear liquid (or filtered if accidently shaken).

(4) *Baryta water* is a saturated solution of barium hydroxide in water, prepared and used precisely as is lime-water; it absorbs carbon dioxide after a similar equation, $Ba(OH)_2+CO_2=BaCO_3+H_2O$. It has the advantage over lime-water in that the barium hydroxide is more soluble (forming about 3% solution), and hence gives a more copious precipitate of the white carbonate.

OTHER DEMONSTRATION METHODS. The above-described method is logical, for both sets of tissue are from the same leaf, in natural attachment to the plant, and under conditions precisely alike except as to the carbon-dioxide supply; and it has also the practical advantage that by stopping tightly the necks of the bottles, they may be kept indefinitely always ready for immediate use, without any change of the chemicals. The method depends of course upon the principle that a bottle of this size contains enough atmospheric carbon dioxide to permit the making of a visible amount of starch. I have also used with success another arrangement giving free access to the atmosphere. It consists simply of two vials cut across the middle and bent inward somewhat at the cut rims so as to hold sticks of, respectively, caustic potash and chalk placed crosswise, the vials being attached to the leaf as shown by Fig. 22; it is, however, less convenient, especially as there is drip from the potash, and less effective for demonstration, than the former method. The method most commonly described in the text-books, in which separate plants or shoots are placed under bell jars which communicate with the atmosphere through tubes containing, respectively, soda-lime and a mechanically equivalent substance, is fallacious in theory; in fact the access of the atmosphere is quite blocked mechanically, and such success as the experiment exhibits is due to the amount of carbon dioxide present in the non-absorbing jar. As a matter of fact this experiment is very effective if only single leaves, with petioles in water, are placed in large sealed jars or bottles, one of which contains soda-lime. Another method is described by DETMER, 54, and another, somewhat elaborate, is given by MACDOUGAL, 229. Another, upon a different principle, was introduced independently by STAHL (Botanische Zeitung, 52, 1894, 129) and by BLACKMAN, Science Progress, 4, 1895, 30); it consists in sealing the stomata of part of a leaf by a thin coating of cocoa-butter and wax, or else by vaseline; but the method is logically defective in that other conditions (transpiration, respiration) are also radically changed.

The use of the carbon dioxide of the air in photosynthesis may also be proven by testing the disappearance of the gas from a closed chamber in which photosynthesis is taking place. This is shown very perfectly by the use of the photosynthometer described a few pages later. But there is another method, used somewhat widely in elementary teaching, making use of the fact that if a flame be burned in a closed space, the carbon dioxide generated, when under 3% strong, puts out the light, which will only burn again after the carbon dioxide is removed. It may be applied as follows. Select a wide-mouth large bottle, and prepare for it a good gas-tight flat stopper having in its middle an opening 1 cm. in diameter closed by another stopper (Fig. 23); attach a candle, the smallest obtainable, at the end of a bent wire; holding the wire with the bend under water, light the candle and place the bottle inverted over it with the mouth in the water. Count the number of seconds until the candle goes out; then remove it and insert under water a large shoot of a green plant; slip a saucer under the whole, lift it out and stand it for a day in bright diffused light; at evening transfer it back to the water, remove the saucer and plant, insert, under water, the double stopper, and invert the bottle with its small quantity of contained water. The wire is now bent of a form such that the candle may be lowered to the center of the jar, and is then pushed through a cork of the same size as that closing the bore of the larger stopper. The smaller cork is now quickly removed, and the candle is lowered into the jar, the cork on its wire tightly closing the opening. The number of seconds is now counted until the flame goes out, a comparison of which with the similar test of the morning gives a crude measure of the amount of the carbon dioxide which has been absorbed. In some ways better than a candle is a tiny gas-flame, which can be made much smaller than that of any obtainable candle, and hence heats and expels the air from the jar to a less degree, besides permitting a more accurate count. The jet can be constructed of glass tubing drawn to a capillary point, the tubing being put through a cork as shown by Fig. 23. In order that the flame may be the same size at the morning and evening tests, the arrangement figured, in which the point may be swung on a movable rubber connection, should be used. The gas-supply must be cut off the moment the flame goes out (by pinching the rubber connection-tube). The experiment is liable to obvious errors (absorption of some carbon dioxide by the water, some gas-exchange at the insertion of the candle, etc.), but nevertheless gives results correct in the main. A method of avoiding the latter error, by lighting the candle inside the jar, is described by MacDougal in his "Elementary Plant Physiology," 93. The experiment is much more effective, especially for younger students, if a second bottle is stood beside the first and treated like it in every respect except that it is covered from

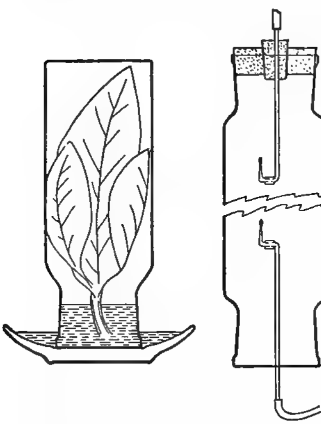


Fig. 23. — Arrangement for a very simple demonstration of the absorption of carbon dioxide by green tissues; $\times \frac{2}{5}$.

The second figure shows use of a gas-flame instead of a candle. Further explanations in text.

light by a suitable hood, and it is even desirable to add a third, in the light without a plant. There are, however, three errors current in connection with this experiment. First it is said in many books that a flame burns the entire 21% of oxygen in the space to carbon dioxide, whereas it never burns more than about 2¼% before the flame is extinguished. The rise of water in the jar is not due to the removal of the oxygen (for that is always replaced by an equal volume of carbon dioxide), but to the expulsion from the bottle of some of the air when heated and expanded by the flame; and the rise is the smaller the less the heat is. And it is especially an error to consider that the burning of the candle at the close of the experiment proves not only that the carbon dioxide has been removed, but the oxygen has been restored. In fact, although the latter is true, the experiment proves only the former.

The student should make sure of his knowledge of the amount of carbon dioxide in the air, upon which he will find matter in Part III of this book. At this point also he should inform himself, through the literature, upon the absorption of carbon by plants in forms other than that of carbon dioxide, and upon the ecological correlations thereof. The mode of absorption of the carbon dioxide and release of the oxygen will be considered later under Absorption.

The use of carbon dioxide in photosynthesis, shown by the preceding experiment, raises the question as to its proportional combination with the hydrogen and oxygen of water to form the photosynthate. Taking into account the known composition of the substances concerned, *viz.*, CO_2, H_2O, $C_6H_{12}O_6$, it will be evident that the most probable, if not the only possible proportions of these substances involved would be expressed by the following equation:

$$6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2.$$

This implies, however, that oxygen is released in the process, a probability so striking as to demand experimental testing:

Is oxygen released in photosynthesis?

This may be determined directly by the analysis of the gas of a closed chamber in which photosynthesis has taken place, but it can be ascertained more simply by testing the gas shown by observation to be given off during photosynthesis by water-plants.

Experiment. Fill a graduated test-tube or cylindrical graduate with water, and support it inverted over a glass jar of water of considerable capacity. Place in the jar, with the cut ends in the test-tube, vigorous shoots of *Cabomba*, *Elodea*, or other plants growing habitually submersed, and stand the apparatus in a moderate (never intense) light for some hours. When sufficient gas has collected in the tube, apply to it an oxygen absorbent, and determine how much, if any, of the gas is oxygen.

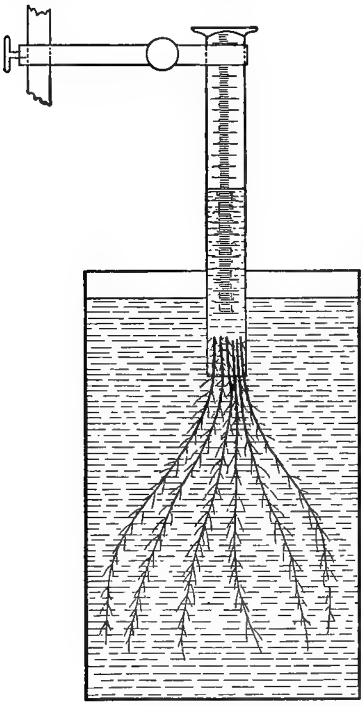


FIG. 24.—EFFICIENT ARRANGEMENT FOR STUDY OF THE RELEASE OF OXYGEN IN PHOTOSYNTHESIS BY WATER-PLANTS; ×⅛.

PRECAUTIONS. The plants should be in growing, not resting, condition. Such plants as give off the gas freely through their cut stems are best treated as described above, but with some others, which give off the gas from their leaves, it is necessary to use a funnel inverted in the water and capped by the test-tube, as shown in all books. In this case, however, it is indispensable that the funnel be not allowed to rest on or near the bottom, and that it shall not fill the vessel, as otherwise the access of carbon dioxide from the atmosphere through the water to the plants is cut off, and gas soon ceases to rise. Also, the gas will be yielded much better if the water is first charged with carbon dioxide from a generator, or from a seltzer bottle, though it will not do to add it in any quantity afterwards, since, as PANTANELLI has shown, if the supply is too large the gas will pass by diffusion through the plant into the test-tube. In making the test for oxygen, the flaming of a glowing splinter often fails because of the lowness of the percentage of oxygen (compare PFEFFER, 1, 331), and it is better to use either the phosphorus test or the pyrogallate-of-potash test, described below. The optimum of light for the process depends somewhat upon the amount of carbon dioxide available, but is always far below full sunlight.

OTHER METHODS. Several ways of applying the above method with convenience and celerity have been described, notably by HANSEN in Flora, 86, 469, and by LINSBAUER in the same, 97, 1907, 263, and by STONE in Torreya, 4, 1904, 17. A self-registering form of this apparatus, liable however to serious practical errors from temperature changes, is described by COPELAND in the Botanical Gazette, 29, 1900, 440. PFEFFER, in his paper earlier cited (page 23, note), describes a method of projecting the plant upon a screen in a way to make the rise of the gas visible to an entire class. A very valuable critical study of this method is given by PANTANELLI in a paper cited below under Literature.

A method very different in principle is the invaluable one of ENGELMANN, based upon the collecting of certain bacteria in places where free oxygen is being released. It is applicable only microscopically, however, though it is invaluable for many special purposes. Directions for its use are in DETMER, 38, and in DARWIN and ACTON, 48. And there are other methods of minor importance (compare JOST, 105).

Still another method, applicable to land plants, is offered by the fuming of phosphorus as oxygen is released in an atmosphere which lacks it. This is arranged by placing a potted plant with the exposed phosphorus in an atmosphere containing only hydrogen and carbon dioxide.

ERRONEOUS EXPERIMENTS. Two erroneous experiments upon this subject are contained in current text-books. The first, against which warning has already been given under Precautions above, is very wide-spread and embodied in recent figures, namely, the use of a funnel, containing water-plants, which either rests upon the bottom of the containing vessel, or even just fits within the walls of the latter. Under such an arrangement, as is easily proven by control experiments, the release of gas presently stops, for the carbon dioxide under the funnel is soon used up, and no more is obtainable. To secure a good result it is indispensable to allow ample room for diffusion of carbon dioxide from the remainder of the vessel into which it is absorbed from the atmosphere, and the larger the vessel the better. The other erroneous experiment, given in several books accompanied by a false illustration, is the one in which leaves of land plants, placed under water, are represented as giving off bubbles of oxygen which rise through the water. It is true that leaves which are enveloped in a film of air do carry on some photosynthesis under water (compare PFEFFER, I, 179), but the amount is so small that it is doubtful if any visible bubbles of oxygen are released, the tiny quantities being taken directly into solution. The bubbles which do collect abundantly upon the leaves of land plants placed in cool fresh water and then stood in the light do not come from the leaves, but from the water itself, for they consist of the dissolved air which is always freed from the water when its temperature is raised, whether this be through standing in the sun or by heating in the dark. It is the same air which collects upon the side of any vessel under these circumstances or upon other solid objects in it; and the bubbles will collect as readily upon dead leaves as upon those alive, and as abundantly in darkness as in light if the temperature be raised as high.

OXYGEN ABSORBENTS AND TESTS. Before attempting to use these absorbents, the student should, to make himself familiar with their action, apply them to the removal of known quantities of oxygen (*e.g.*, as in air) from closed graduated tubes.

(1) *Potassium pyrogallate*, in alkaline solution, is for most purposes the best means for removing oxygen from a confined space, and thus, inci-

dentally, forms a good quantitative test of its presence. It is made by mixing pyrogallic acid in solution with a surplus of caustic potash, when the pyrogallate is formed thus: $C_6H_3(OH)_3 + 3KOH = C_6H_3(OK)_3 + 3H_2O$. This solution is an eager absorbent of oxygen (forming a soluble dark-brown substance, $C_{20}H_{20}O_{16}$), and, of course, owing to the presence of surplus caustic potash, of carbon dioxide. The most diverse proportions of the constituents have been recommended by different workers, from the .25 g. (solid) pyrogallic, and .6 g. caustic potash in 10 cc. water (*viz.*, 1 to 2.4 to 40), found by WEYL to be the optimum (Ann. der Chemie, **205**, 255, and Ber. d. d. Chem. Ges., **14**, 2659 and 2667), to the 5 g. pyrogallic acid, 120 [*sic*] g. caustic potash in 95 cc. water (*viz.*, 1 to 24 to 19), of HEMPEL ("Methods of Gas Analysis," 115). The WEYL solution evolves carbon monoxide (about 1/30 of the oxygen absorbed) if the gas contains over 28% of oxygen, but the HEMPEL solution quite prevents this (CLOWES in Proc. Chem. Soc., 1895, 200), which is a reason for the use of this strength. I have myself somewhat thoroughly tested the different solutions from the present point of view, and find that one consisting of 1 part pyrogallic acid to 5 parts caustic potash to 30 parts of water gives an optimum between absorptive power and convenience in preparation; it will absorb about 10 times its own bulk of oxygen (one molecule pyrogallic acid with three of caustic potash absorbs three atoms of oxygen, that is, 1 gram pyrogallic acid absorbs nearly one-third of a gram of oxygen; BERTHELOT, Comptes Rendus, **126**, 1898, 1459), though in practice it is best to allow only half that quantity or less (one-fourth recommended by HEMPEL). The absorption should take place at a temperature above 15° (below which it is slow), and, if the solution is constantly agitated or flowing, only about three to five minutes is required, though much longer is needed if the solution is still. Since the solution spoils almost immediately in air, and in a few hours in light, it should be made up just before using, which may conveniently be done by mixing at the moment of use equal parts of a 1 in 15 solution of freshly made pyrogallic acid with a 5 in 15 solution of caustic potash, made up long enough before use to have lost its heat and air. Nor should the experiment be left standing for hours, since the solution may ultimately give off carbon dioxide. The solution may best be applied to the gas by use of the reagent tubes described on the next page.

(2) *Phosphorus.* For some purposes phosphorus is a more convenient absorbent of oxygen than is pyrogallate of potash, but it cannot be used where living tissues are in any way concerned, is slower in its action, and is more dangerous to use, both because of the bad burns it can inflict, and also because of its tendency to ignite in the air. It is best employed in the slender sticks supplied for this especial purpose, which are used in a wire cage held on a wire support; this is inserted into a chamber, or reagent tube over water, and then is surrounded for some time, at a temperature above 15°, by the air to be analyzed. During this time, as well as at all other times, the phosphorus should be in darkness to prevent formation of an amorphous coating over it. In absorbing oxygen white fumes are given off, through formation of anhydride of phosphorous acid, P_4O_6, and this immediately dissolves in the water after the equation $P_4O_6 + 6H_2O = 4H_3PO_3$.

METHODS OF GAS-ANALYSIS. Of these the most important by far in Plant Physiology apply to carbon dioxide and oxygen. Somewhat elaborate but very exact methods have been described by HEMPEL and WINKLER (their books: DARWIN and ACTON, 6; MACDOUGAL, 235), by TIMIRIAZEFF (his micro-eudiometer, DARWIN and ACTON, 45), by BONNIER and MANGIN (MACDOUGAL, 258, though the accuracy of this apparatus has been questioned by PANTANELLI in a paper cited under Literature). I have myself devised a method, much simpler than any of the above, but amply efficient for all except precision work. It makes use of the usual absorbing chemicals described elsewhere (pages 98 and 103), but applies them by means of reagent tubes illustrated in the accompanying figure (Fig. 25). The gas to be analyzed is collected or brought into a graduated tube,—over water if the absorption by the water is negligible, otherwise over mercury. Then a reagent tube of the same diameter, sealed at one end and provided at the other with an extension of stout rubber tubing, is filled to near the top of the rubber with either the caustic potash solution, or the potassium pyrogallate, as need may be, and is sealed by a screw clamp as in the figure. It is then placed under the water (or mercury), all air is carefully squeezed from the tube above the clamp, and the rubber is slipped over the open end of the graduated tube, which it should grip firmly. The whole is then lifted from the water, the clamp is opened, the combination is inverted, and the liquid is allowed to flow back and forth several times from one tube to the other, when it will completely absorb any carbon dioxide (or oxygen) present. The combination is then held upright until the liquid has all settled downward, when the clamp is closed. Then the combination is slipped again under water, and the rubber tube is pulled off, when the atmospheric pressure will instantly force up the water to the exact extent of the gas absorbed, permitting the amount to be read off directly. The corrections for capillarity and vapor-tension may be ignored in demonstration, though in exact work they would be taken into account. Barometric pressure obviously can introduce no error, but temperature changes must be compensated either by calculation or by reading the graduated tube both before and after the test, while a stream of water of constant temperature is flowing over it. A source of error to be guarded against is the possible leakage of air into the tubes as the gas is absorbed and the internal pressure reduced. This can be prevented by tight-fitting tubing, especially if the ends be tightly wrapped

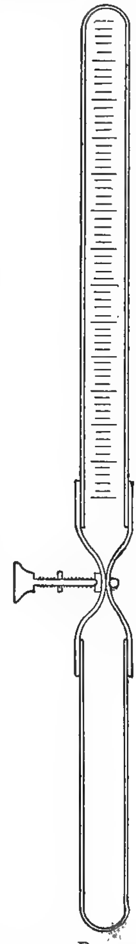


FIG. 25.—REAGENT (LOWER) AND GRADUATED TUBES, WITH RUBBER CONNECTION AND CLAMP; $\times \frac{2}{5}$.

with tire tape; and it can be compensated by carrying on the entire operation under water, the leakage of which does not matter. Both graduated and reagent tubes may be made of any desired size, but they are more manageable and equally accurate if made rather small, a practically convenient size being that shown by the figure (holding about 8 cc.), which permits the absorbing solutions to be made up from sticks of commercial caustic potash directly in the tubes. Thus in the reagent tube figured, the caustic potash may be made up by (*a*) dropping 2 cm. (*viz.*, grams) of a commercial stick into the tube; (*b*) filling it to above the clamp with water; (*c*) clamping it until the stick is dissolved; (*d*) opening it to allow escape of the air collected from the stick; (*e*) clamping it tight again until needed. If kept closed from the air it keeps good indefinitely, and if account is kept of the amount of carbon dioxide absorbed, it may be used up to its limit, which is approximately 800 cc., though practically 200 to 400 (page 98) of that gas. For oxygen absorption half the quantity of caustic potash and water is to be used, and to it at the moment of using is to be added an equal amount of 1 part in 15 (by weight) solution of pyrogallic acid, which should be kept closed from air and from strong light. It will absorb about 60 cc. of oxygen, say 25 for safety. In keeping record of the absorption it must be remembered that the solutions are diluted with every use to an amount varying with the amount of water in the graduated tube, an error to be guarded against. These tubes may be adapted from laboratory materials, and they are to be supplied ready for use among my normal apparatus (page 46).

The student should now make sure of his knowledge of the gases available to water-plants, and their respective solubilities, on which there are data in Part III.

So far the study of the gases concerned in photosynthesis has been purely qualitative. But the scientific spirit demands that everything possible shall be made quantitative. To this end we turn again to scrutinize the equation, which has proven true so far and which may well be called, conventionally at least, *the photosynthetic equation, viz.*, $6CO_2+6H_2O=C_6H_{12}O_6+6O_2$. Viewing this equation from the quantitative point of view, it seems evident that the two gases concerned are equal in volume, a matter which calls for experimental study.

Are the carbon dioxide absorbed and the oxygen released in photosynthesis equal in volume?

This may be determined by keeping lighted green tissues for a time in a closed chamber, and then analyzing the enclosed gas for any increase of oxygen and any diminution of carbon dioxide known to have been present at the beginning of the experiment. Since the carbon dioxide is present in the atmosphere in an amount impracticably small for ordinary analysis, advantage may be taken of the fact that plants will thrive for a time in an atmosphere containing a much larger percentage, even up to 10 per cent of that gas.

EXPERIMENT. In the chamber of a photosynthometer place a green shoot of appropriate size of a vigorous small-leaved plant (*e.g.*, Heliotrope, *Ficus repens*). Supply to the enclosed atmosphere enough carbon dioxide to make a 10 per cent mixture, and place the whole in strong diffused light for some three or four hours; then close the communication with the gas-tube, and at leisure determine the composition of the gas as to oxygen and carbon dioxide.

PRECAUTION. It is best in this, as in all experiments where plant tissues are exposed behind glass, not to permit full sunlight to fall upon the tissues, since this is likely to cause abnormal heating and injury in the absence of ventilation; and besides, in the present case, the resultant gas expansion would be likely to force off the stopper of the instrument.

PHOTOSYNTHOMETERS. Of these (though not under this name) several forms have been invented, of which the most exact is PFEFFER'S (Arbeiten des botanischen Instituts in Würzburg, 1, 1871, 9; in synopsis by DETMER, 41, and by DARWIN and ACTON, 41). Though accurate, the apparatus is not readily obtainable, and the manipulation is rather cumbersome. I described a simplified modification of it in the first edition of this book, 93, while STONE has described yet another way in Torreya, 4, 1904, 1. Much more satisfactory in every respect, however, is the instrument which I have described in the Botanical Gazette, 41, 1906, 209, and which is among my normal apparatus (page 46), and figured herewith (Fig. 26).

The instrument consists essentially of a pear-shaped plant chamber set in a firm iron base, a graduated measuring-tube with a small stop-cock at the upper end, and a connecting stopper furnished with a stop-cock of considerable bore. The total capacity of the apparatus when closed is exactly 102 cc., of which the 2 cc. is for a shoot and 100 cc. for the gases concerned. The proper amount of shoot is provided by selecting a small-leaved plant and pushing a branch down into a measuring-glass until it displaces exactly 2 cc. of water; the water-level is then noted on the stem, which is cut at this point under water, the shoot being later, when shaken free from water, placed upright in the chamber. We now add some selected percentage of carbon dioxide to the apparatus in the following way. The measuring-tube, with stop-cock closed, and handled always by the top only to prevent volume changes of its gas by heat of the hand, is inverted and filled with water of room temperature up to a figure of the graduation expressing the selected percentage, for the tube is graduated in cubic centimeters, which are, of course, percentages of the total gas capacity of the apparatus. The stopper is then placed on the tube and its stop-cock closed; its hollow is filled with water and the whole is inverted in a pneumatic trough (or equivalent dish of water) which has been standing in the laboratory long enough to take the temperature of the air. The lower stop-cock is then opened and carbon dioxide from a generator is allowed to enter the tube, either from below or, as is most convenient, through the top of the tube. The admission of the gas may be perfectly controlled by cautious manipulation of the upper stop-cock, and this is closed at the moment when the water has been wholly driven out to the bottom of the bore of the lower stop-cock, which point is held exactly at the water-level. The lower stop-cock is then closed, and the combination, which now contains exactly the desired percentage of carbon dioxide, is lifted from the water, shaken free from all adhering drops, and placed in position on the chamber. To prevent compression, and therefore the presence of too great a quantity of air, in the chamber when the stopper is pushed into place, tiny holes (visible in the figure), matching in stopper and chamber neck, allow free release of such pressure, and the chamber is perfectly sealed by twisting the stopper a little. The lower stop-cock is then opened, permitting the carbon dioxide of the tube to diffuse into the chamber, a process hastened by its gravitational flow; and care should be taken to jar down any water bubble which may tend to close the passage from tube to chamber. The apparatus now contains obviously 2 cc. of plant and 100 cc. of gas, of which a known percentage (say 5, 8, or 10) is carbon dioxide, and the remainder is air. The instrument is now placed in a bright light, not direct sunlight, for three or four hours; then the lower stop-cock is closed, as shown in the figure, shutting into the tube a sample of the gas of the chamber at the close of the experiment. The analysis of this gas can be made at leisure, and is accomplished thus. The

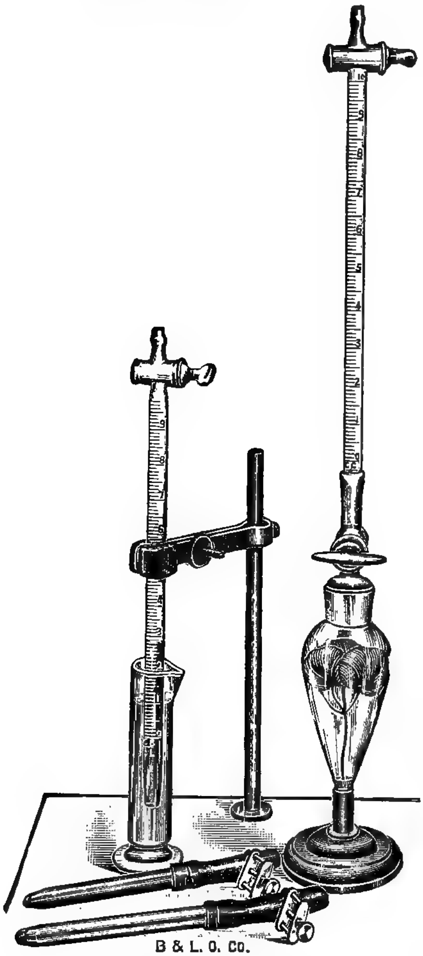


FIG. 26.—PHOTOSYNTHOMETER; ×½.
Explanation in text.

stopper and tube are lifted from the chamber and placed upright in the pneumatic trough, deeply enough to allow the stopper to be taken off without the admission of air to the tube. The zero mark of the tube is then brought exactly to the water surface; the upper stop-cock is cautiously opened, permitting the water to rise slowly to the zero mark, when the stop-cock is again closed, shutting into the tube exactly 10 cc. of the gas to be analyzed. First the quantity of carbon dioxide in the tube is determined, which is accomplished by aid of the reagent tube described on page 105. Next a determination of the percentage of oxygen present is made by another reagent tube, containing pyrogallate of potash, used in the same manner. Some slender vessel is then slipped under the measuring-tube, which is removed and supported for observation, as shown in the figure. Corrections are to be treated as described earlier (page 105). The measuring-tube should always be washed quite free from the reagents at the close of every analysis, and the ground joints should be kept slightly lubricated by the usual wax.

In studying the process with beginners, the demonstration is the more striking and conclusive to them if a second instrument is set up like (and beside) the first, but covered completely from light; while even a third, like these two except that it has no plant, may advantageously be added. When interpreting the final results it must be remembered that the experiment is started with only 90% (if 10% carbon dioxide is used) of air, that is, 72% of nitrogen and 18% of oxygen.

It is quite possible to adapt a photosynthometer from a graduated tube, the bulb of a calcium-chloride tube, and a rubber stopper, as shown by the accompanying figure (Fig. 27), the principle of its use being nearly identical with that of the instrument just described. Its capacity must of course be accurately determined; the proper quantity of carbon dioxide is put into the graduated tube, which is sealed with the finger until pushed into the bulb, the small opening of which is immediately afterwards sealed with tire tape.

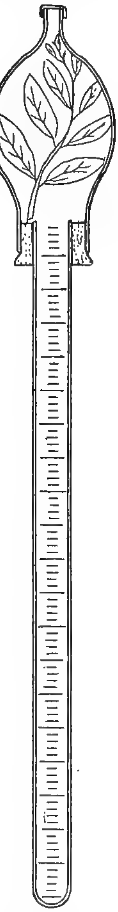


FIG. 27.—SIMPLE PHOTOSYNTHOMETER; ×½. Particulars in text.

In connection with the use of a 10% carbon-dioxide mixture in the preceding experiment, the student should look into the current statements as to the strengths of that gas green plants can endure, and also into the supposed optimum strength, upon which latter point he should consult the critique of optima by BLACKMAN in Annals of Botany, **19**, 1905, 281.

The exactness of the photosynthetic equation demonstrated by the preceding experiment calls to attention the fact that, as in all such equations, when certain quantities are known the others can readily be determined. The student should now, using the data given in Part III, calculate in grams or other desired units, *how many grams of the photosynthate can be formed from a given number of grams of carbon dioxide or of water, how many grams of oxygen are released in the process, how many grams of carbon dioxide or of water are needed to form any given number of grams of photosynthate or to release so many grams of oxygen, and how much atmosphere must be emptied of its carbon dioxide to form any given quantity of photosynthate.* The student should make these various calculations, using definite quantities, and should practice the calculations until he can make them with ease, certainty, and even pleasure.

There is yet another fact, of the utmost importance, shown by the photosynthetic equation, namely, that either the carbon dioxide or the water, or perhaps both, are dissociated in the process. Since these are extremely stable substances, they require a large amount of energy to dissociate them, and the question arises as to the source of this energy. Here one at once recalls the constant association of the process with light, as well as the stoppage of a part of the light energy by the chlorophyl, shown by the black bands of its absorption spectrum. These facts suggest that the particular energy which does the photosynthetic work consists of those principal rays of light which are absorbed by the chlorophyl. So important a matter requires experimental study, which formulates an important problem as follows:

Are the rays of light absorbed by chlorophyl capable alone of doing photosynthetic work?

This may be determined by supplying to a green leaf rays spectroscopically equivalent to those absorbed by the chlorophyl, either withholding all others, or else, and better, supplying them as a control, and noting whether photosynthesis takes place.

EXPERIMENT.—In four good vials, some 1 cm.×6 cm. in size, place pure-color solutions made up, by aid of the spectroscope, to match the black bands of the chlorophyl spectrum as closely as possible, *viz.*, *red* from the dye "scarlet," (orange-yellow seems unattainable in one liquid), *green* from a mixture of ammoniacal sulphate of copper with potassium chromate, and *blue-violet* from ammoniacal sulphate of copper. Add, as a control, a vial of distilled water. Place the corked vials side by side on a glass plate (Fig. 28), separated from one another and from the plate by tin-foil, except that under each vial is left an open slit 3 mm. wide and the length of the vial; on the under side of the plate add tin-foil with exactly matching slits, and then place the plate on the leaf of a plant kept previously a day or two in darkness, following the normal light-screen method (page 87). Expose the leaf to bright light for two or three hours, then blanch it and apply the iodine test.

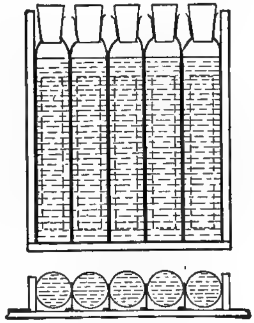


FIG. 28.—ARRANGEMENT OF VIALS UPON A GLASS PLATE TO FORM A PURE-COLOR SCREEN; $\times\frac{3}{8}$. The heaviest lines represent tin-foil.

PRECAUTIONS. The solutions must be made up with the spectroscope, and should be such that each transmits the full intensity of its own light, but no other rays. The vials should be not quite full, and to prevent the forcing out of the corks under the sun's heat, there should be a fine thread between each cork and neck, which permits some adjustment of pressure. The vials may be attached to the tin-foil (which is itself gummed to the glass) by drops of shellac; and it is well to surround them by wooden strips as in the figure (Fig. 28). The colors may be preserved in their vials for future use. Several sources of error are present, *i.e.*, the difficulty of making the colors spectroscopically accurate, the different heating effect of the different colors tending to promote translocation in different degrees, and a very probable more rapid translocation from under the blue than from under the red. The form of the vials concentrates the light upon the leaf, hence partially compensating the great absorption.

OTHER METHODS. This important subject may be approached in any one of several ways. One of the best known is that used by SACHS, in which double-walled glass bell jars, filled between the walls with colored liquids, are placed over similar plants, the amounts of photosynthate being afterwards determined by the starch test. The jars are expensive and the method somewhat slow and cumbersome, and it introduces the individual-difference error inseparable from the use of different plants. In place of the bell jars, MACDOUGAL (124) uses concentric glass cylinders. A very different method consists in the counting of the oxygen bubbles given off by water-plants placed behind colored screens or in different parts of a spectrum; it is somewhat difficult of application, but directions are contained in the accessible books (DARWIN and ACTON, 35; DETMER, 36). Another method, in some respects ideal, is that of TIMIRIAZEFF (described in Comptes Rendus, 110, 1346), whereby the solar spectrum itself, held in a fixed position by a heliostat, is projected directly upon a leaf in a dark chamber, the amount of photosynthate formed being determined by the starch test. The spectrum method has been used by others also (JOST, 126).

It would seem at first sight that monochromatic screens could be constructed from plates of colored glass or of gelatin, or of films of collodion on glass; and certainly such screens would be much more convenient in manipulation than any liquid colors. But unfortunately the obtainable colors of glass or gelatin are shown by the spectroscope to be of mixed composition; only a few of them (including, however, a good red) transmit their own colors alone, which shows how unsafe a guide to the composition of colors is the eye unassisted by the spectroscope. Further, with such screens it is very difficult to secure not only precisely the right colors, but also just the correct intensity. A range of colors suitable for such screens, or filters, is given by MACDOUGAL, 131, by DAVENPORT, 157 (also 158), and by WOOD, "Physical Optics," 10.

The result of the foregoing experiment identifies, though in a somewhat crude way, the energy used in photosynthesis, and shows its general relation to the dark bands of the chlorophyl spectrum. It throws no light, however, upon the exact mode of transformation or application of the energy to the especial work of photosynthesis, *viz.*, the dissociation of the substances concerned. The student should now inform himself upon our present knowledge and suppositions upon the subject; and he should make sure he understands *the significance of the energy transformation in photosynthesis, viz., the dissociation of very stable simple substances through kinetic energy of light, and the formation of an unsaturated or oxidizable synthate, with the consequent storage of latent or potential energy.* He is here dealing with the most important single process in the entire range of the chemistry of organic materials. He should also consider here the proportion of the sunlight energy used in photosynthesis, on which there is a most valuable paper by BROWN in Nature, **71**, 1905, 522.

But there are certain minor points also,—why photosynthesis should occur under light waves having such diverse wave-lengths as red and blue, and whether it is possible to produce photosynthesis outside the living protoplasm,—on which the student should seek information from the proper sources. He should also understand the photosynthetic graphs of ENGELMANN, of REINKE, and of KOHL, their meaning and the mode of their construction.

Photosynthesis, with its needs for exposure of much green surface to light, for free access of carbon dioxide, for a supply of water, for translocation of its products from the place of formation, becomes the center of very important ecological features which the student should make sure that he clearly understands. He should embody his knowledge in a brief exposition, with illustrative diagrams, making plain (*a*) the structures which are adapted to the demands of the actual physico-chemical process; (*b*) the compromises of these structures with those adapted to other necessary processes of the plant; (*c*) the features, protective or adjustive, which relate these to average or ordinary external conditions; and (*d*) the modifications resulting from adaptation to special habits.

TERMINOLOGY AND CONCEPTIONS OF PHOTOSYNTHESIS. The student will have noticed early in his study of the literature of this subject that most European books name the process "assimilation" or "photosynthetic assimilation," while American works call it "photosynthesis." The former is an old term coming down from the time when the process was supposed to be a part, and the principal part, of that which in plants corresponds to assimilation in animals. We now know that the process is an entirely distinct one, found only in green plants, with nothing like it in any way in animals, while plants do also have a process of assimilation physiologically equivalent to assimilation in animals. Hence it is better from every point of view to restrict the older term to its proper function, and apply to this process the very appropriate and distinctive term photosynthesis, invented by C. R. BARNES in 1893 in the form photosyntax, and changed later to the present form. The process is also sometimes called popularly "food manufacture." (History of the word by BARNES in Botanisches Centralblatt, **76**, 1898, 257.)

PHOTOSYNTHETIC QUANTITIES. As to the amounts of photosynthate made, SACHS found that herbaceous plants out-of-doors in summer under most favorable conditions made 1.882 grams of photosynthate per square meter per hour, though BROWN and BLACKMAN have found much smaller quantities. Taking all the data together, we may accept for plants under best conditions out-of-doors 2 grams as an extreme, with 1 gram as a mean, giving a conventional constant (page 22) of 0–1–2 gm^2h. Studies by my own students have shown that greenhouse plants under the best of conditions in winter approximate to one-half the quantities of those out-of-doors, that is, 0–.5–1 gm^2h. As to the amounts of the various substances concerned, the student will of course work these out exactly by use of the photosynthetic equation, replacing molecular weights by gram weights. He will then see that conventional constants may be accepted as follows: to form one gram of photosynthate requires 1.5 grams, or 750 cc., of carbon dioxide, which is the quantity contained in 2500 liters of air. In other words a square meter of leaf in making one gram of photosynthate uses all the carbon dioxide of a column of air over it 2.5 meters in height.

As to the energy stored, this will be the exact reciprocal of that released in respiration, as considered under that process later. As to the amount utilized of the energy of sunlight falling upon the leaf, BROWN (Nature, **71**, 1905, 522) has shown that this is less than 1%; the fate of the remainder will be noted later under Transpiration Quantities.

LITERATURE OF PHOTOSYNTHESIS. This is summarized down to 1900 by PFEFFER, and there is a later synopsis by JOST. Some special papers of particular value to students in this course, in addition to those already mentioned in the preceding pages, are those by BROWN in Nature, **60**, 1899, 474, by TIMIRIAZEFF in Proceedings of the Royal Society, 1903, 421 (good summary in Botanisches Centralblatt, **96**, 1904, 529), by BROWN and ESCOMBE in Proceedings of the Royal Society, **76**, 1905, 29, and by BLACKMAN and MATTHAEI in the same journal, **76**, 1905, 402, and by BROWN in Nature, **71**, 1905, 522. There is also a very recent summary of our knowledge of the subject by CZAPEK in Progressus Rei Botanicæ, **1**, 1907, 468.

2. CHEMOSYNTHESIS.

In his study of carbon fixation through photosynthesis, the student must be impressed by the importance of the kinetic energy of light in the process, whence the question is natural, can the process take place if the energy is supplied in some other manner, and is any other manner known? In fact a case is known in certain Bacteria which utilize chemical energy derived from oxidation of mineral substances, a true process of Chemosynthesis. The subject is one of extreme experimental difficulty, and the student must seek information upon it through the literature, in which there is a very satisfactory treatment by PFEFFER, **1**, 361. Furthermore it is altogether probable that much. if not most of the energy used in synthesis of proteids, next to be considered, is chemical, so that proteid synthesis is no doubt largely Chemosynthesis. Theoretically both Thermosynthesis and Electrosynthesis are conceivable, though they are not known to occur.

3. SYNTHESIS OF PROTEIDS.

In his study of the chemical composition of the photosynthate, the student must have come into contact with references to a very important class of plant substances, of which Protoplasm is largely a mixture, called proteids, whose most notable characteristic is the possession of nitrogen, sulphur, and phosphorus, in addition to the carbon, hydrogen, and oxygen of the photosynthate. It becomes important now to investigate the mode of synthesis of these proteids, a subject of all the more consequence since it involves economic interests of magnitude. Unfortunately our knowledge of the subject is very defective, and its experimental study is correspondingly difficult, but the student must follow it even if only through the literature.

We consider first the nitrogen of the proteids, and the first question naturally relates to the source of supply. Both general probabilities and the analogy of photosynthesis would lead us to expect to find the source of the plant's nitrogen in the abundant store of the atmosphere. Yet as ample nitrogen in the form of nitrates is present in the soil, that possible source cannot be ignored. The matter can readily be settled by experiment, which involves this problem:

Do plants obtain the nitrogen of their proteids from the free nitrogen of the air, or from the combinations in the soil?

This may be determined, indirectly, by so growing two sets of similar plants that both have full supply of atmospheric nitrogen, while one is supplied with combined nitrogen through the roots and the other lacks it; then the comparative growth of the two sets must show whether or not the soil nitrogen is needed. This can most readily be effected through water-culture.

Experiment. On each of the covers of two water-culture vessels place ten Oats germinated by the method described later under Growth; fill one vessel with a standard water-culture solution, and the other with the same, excepting that the nitrogen salt is replaced by a calcium salt. Give favorable conditions for water-culture growth, and compare their relative progress; finally compare the relative dry weights.

NUTRITIVE SOLUTIONS. Of these a considerable number have been recommended, all no doubt good, since the combinations matter little so long as the necessary substances and quantities are present. I have found that given by DETMER, 2, practically one of KNOP'S, to be admirable for all ordinary uses. It consists of 1 g. calcium nitrate and .25 g. each of potassium chloride, potassium phosphate, and magnesium sulphate, all dissolved in a liter of distilled water to which is added a few drops of ferric chloride. The solution without nitrogen is made up in the same way except that calcium sulphate replaces the calcium nitrate. Doubtless it would be better, in order to prevent the development of organisms in the solution while stored, to dissolve the salts in 50 cc. of distilled water, and dilute as needed. For other solutions and various accessory matters, consult DETMER, 1, or DARWIN and ACTON, 58.

Culture minerals put up compressed in tablets, said to be very convenient in use, have been introduced by EDWARD F. BIGELOW of Stamford, Conn., from whom they may be obtained at a low price. He has described their use in the Nature Study Review, 1, 1905, 69.

WATER-CULTURE VESSELS. For the most efficient work with water-culture, very large vessels, and a number of special precautions, are necessary, upon which full information may be found in the works of DETMER and of DARWIN and ACTON, above cited. For our present purpose, however, and for most simple demonstrations, where the plants are not to be carried through a complete cycle back to the seed, very much simpler arrangements are ample. Thus small wide-mouth bottles, covered with black paper and fitted with corks cleft at the margin to hold the seedlings, are used with success in the United States Department of Agriculture, where also a system has been developed of germinating the seedlings upon paraffined or gutta-percha netting supported by corks on water (LIVINGSTON, The Plant World, 9, 1906, 11, and later information). I have, however, found the following simple arrangement wholly satisfactory. Take two of the largest procurable plain glass tumblers, and cast for them covers of hard paraffin blackened by admixture of lampblack. This can be done best by use of a mould turned for the purpose from wood, and covered with glycerin to prevent adhesion of the paraffin, though the mould may be extemporized from cardboard, or even dispensed with in favor of the top of the tumbler itself into which the melted paraffin can be poured upon the water, though this is less satisfactory. The cover should be some 5 mm. thick, and have a projecting rim as shown by the accompanying figure (Fig. 29). Holes may now be bored by a hot iron and made of just the size and form to hold the seeds firmly upright. The tumbler is now surrounded by a readily removable shell of opaque paper, which should darken the tumbler, to prevent development of Algæ. The seeds may most conveniently be germinated in a saucer germinator (described later under Growth), though they will germinate in place on the cover almost equally well if soaked and covered temporarily by wet filter-paper. For our present purpose the seedlings once started need no further attention; but an occasional change of solution will permit their continued healthful growth. For some plants an occasional aeration of the water is desirable (page 49). They should be stood in a good light, but not direct sunlight, but it is well to keep them darkened and under a bell jar until the seeds germinate.

If, after this experiment, the student has any doubt as to the ability of plants to synthesize proteids from the photosynthate and nitrogen-containing salts, he may convince himself by an experiment detailed by DETMER (58), and which he will himself try later in connection with Fermentation.

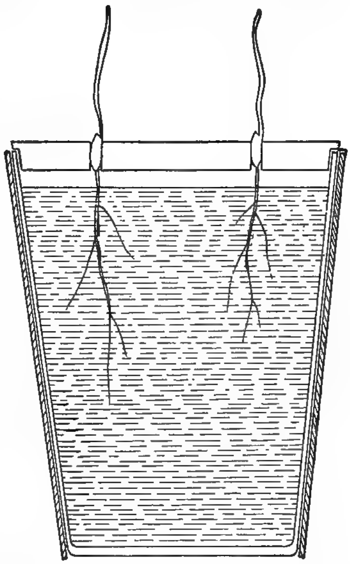


FIG. 29.—WATER-CULTURE VESSEL; $\times \frac{2}{5}$.
It is a tumbler, with top of hard paraffin and wrapped in felt paper.

Turning next to the proteid formed, analogy with photosynthesis would imply that some one simple form is first made from which the others are derived. The student must seek to learn, through the literature, *whether there is a basal proteid, what intermediate stages are known or supposed to exist in its formation, where it originates in the plant, and what its energy relations are, whether chemosynthetic or photosynthetic.* And he should express his results in a proper exposition of our knowledge of these important matters. Here also he should consider whether there is any known excretion of nitrogen-holding compounds, as occurs so abundantly in animals.

It is to-day a matter of common knowledge that there is one family of plants, the *Leguminosæ*, which have unique relations with aerial nitrogen through the colonization on their roots of nitrogen-fixing bacteria. The student should now inform himself, through the literature, upon our present knowledge of this subject, and upon the efforts being made to turn it to practical account. And he will be aided much in an understanding of the matter by some practical work, which he may do along the following lines:

SUGGESTED EXPERIMENTS. Procure two pots of soil from ground known to produce good crops of green peas, and thoroughly sterilize one of them by steam (to kill all bacteria). Then plant in each the same number of good seeds of green Peas, give them favorable conditions for growth, and observe results. Finally examine the roots for the presence of nodules. If practicable apply bacteriological examination of these.

Procure two similar pots of soil, and thoroughly sterilize both. Then to one add a culture of Pea-form of bacteria such as is issued for experimental purposes by the Department of Agriculture; plant in both equal numbers of similar Peas, give proper conditions for growth, and observe results.

The action of these bacteria raises a question as to the presence of such forms in the soil, and this in turn suggests an inquiry as to *the various methods by which soils receive their supplies of combined nitrogen, that is, how soils are nitrified.* Upon this very important matter, so important from an economic as well as a scientific standpoint, the student should now acquire information and express his results in a proper exposition.

Finally, under this subject, we have to take account of those curious cases in which some plants obtain their nitrogen in the form of compounds from other plants. This is done to some extent by all parasites, but it is especially peculiar to the Insectivorous Plants, whose ecology the student should here consider. This animal-like acquisition of combined nitrogen brings up also the consideration of the acquisition of combined nitrogen by animals, their sources of supply, their conservation or otherwise of it, and this the student should also consider.

So much for the absorption of nitrogen; we turn next to the sulphur and phosphorus. Our knowledge of their utilization, however, aside from the mere fact that they are derived from the sulphates and phosphates of the soil, is extremely scanty; and the experimental difficulties of the subject are so great that the student must be content to work it up through the literature.

NOMENCLATURE OF PROTEIDS. As this work is in press, there has appeared an important report, by a committee of chemists, upon proteid nomenclature (Science, 27, 1908, 554), the most striking recommendation of which is the abandonment of the form proteid in favor of protein, a usage which will no doubt soon come to prevail.

LITERATURE OF PROTEID SYNTHESIS. The subject of proteid synthesis is summarized in the works of PFEFFER and of JOST. There is also matter of importance in LOEB'S "Dynamics of Living Matter" (New York, Columbia University Press, 1906), and incidentally some notes of interest in BARNES' address, mentioned later under Respiration. Upon nitrification of soils there is an admirable address by WARD in Nature, **56**, 1897, 455 (and earlier in Science Progress, **3**, 1895, 251), and there is an excellent discussion in Chapters X and XI of FISCHER'S "Structure and Functions of Bacteria" (translated by JONES, Oxford, Clarendon Press, 1900). Upon water-culture, in addition to the works cited, the latest important paper is by SCHIMPER in Flora, **73**, 1890, 207.

4. CONVERSION.

(ASSIMILATION PROPER, INCLUDING DIGESTION.)

Having traced the synthesis of the basal carbohydrate and (so far as known) of the basal proteid, it is next essential to trace their conversion into the great variety of organic substances of diverse special functions and meanings which they form, a transformation effected as a rule with slight or no changes of energy relations. The study is largely one of organic chemistry, involving, in its advanced and quantitative phases, special manipulation of great difficulty, but in its simpler and qualitative phases, practical manipulation which is time-consuming rather than difficult. It will be a great aid to the student, giving the subject a far clearer and more nearly objective meaning, if he can come into personal contact with it through some of the latter type of experiment, which he can do by aid of the following:

SUGGESTED EXPERIMENTS. Prepare, examine macroscopically and microscopically, apply the more prominent tests to, and observe the more prominent reactions of the common and important carbohydrates and proteids of the plant, following as a guide the excellent directions in DETMER, 258, 293, or of DARWIN and ACTON, 249. There are also good directions in SNYDER'S "Chemistry of Plant and Animal Life" (New York, Macmillan, 1903). There should also be included a polariscopic study of starch and other bodies of definite structure; a polarizer and analyzer arranged for the microscope are obtainable from ZEISS at a cost of about 40 marks.

The student should now make a classification of the substances found in plants, so tabulating them as to show (*a*) *the principal classes or groups, with their names and typical formulæ;* (*b*) *the chemical relations of the groups to one another in order of increasing complexity;* (*c*) *places of their occurrence;* (*d*) *their meaning in the economy of the plant, whether working materials, skeleton substance, reserve foods, special secretions of ecological significance, or by-products.*

Of the substances in the above classification, some are soluble and some are insoluble in water, the only transporting medium of the plant; yet some of the latter, *e.g.*, starch, are known to be transported, and hence must be converted for the purpose into a soluble form. The precise physics of the translocation will be considered later (under Transport), but it is necessary here to consider how the solution is effected. As a type we may take the solution or hydrolysis (digestion) of starch, well known to be effected by diastase, which presents a study as follows:

What phenomena are exhibited in the action of diastase upon starch?

Experiment. Prepare a diastase solution by germinating some 20 g. of barley until the sprouts are one-third the length of the grain; then grind it up finely in a mortar (to break the cells), add 100 cc. of water, let it stand, stirring at intervals, for one or two hours, then filter. Prepare a starch paste by adding 1 g. potato starch to 100 cc. water and heating to boiling-point. Mix together 5 cc. of the barley solution and 25 cc. of the starch paste. When mixed place a few cc. in a test-tube, add a few drops of iodine, and note the color; repeat this test at intervals of fifteen minutes, until there is no change of color. Keep in a warm place, the warmer the better, so long as not in excess of 60°.

The test may also be made with diastase (obtained from a chemical supply company) dissolved in water, or by using dry malt in place of the germinated barley.

Diastase is an example of the very important and interesting substances, the enzymes, and upon their chemical nature, known or supposed mode of action, various classes and effects, the student must now inform himself through the literature, noting especially Green's monograph. The hydrolysis of insoluble substances by ferments or enzymes inevitably raises the question as to the method by which the reverse process is accomplished, *viz.*, the formation of the insoluble substances from their corresponding hydrolytes, and upon this matter the student should inform himself. This will bring him to a consideration of accumulation of reserve substances and the closely related subject of secretion of substances of special function, to which also he must here give attention.

Closely correlated with this subject of conversion is that of the function of the various minerals essential to the metabolism of the higher plants, but the state of our knowledge of this subject is such that little can be said of it in this connection, and it may best be treated later along with the phenomena of Absorption.

Another related matter which should be considered concerns any corresponding processes in the animal kingdom, and especially the changes in the various classes of plant substances when absorbed and digested by animals.

THE IDEA AND TERMINOLOGY OF PLANT FOOD. As happens so often in science, the advance in our knowledge of plant nutrition has introduced difficulties with the older terminology. The term *plant food* was originally applied to the minerals, water, and gases absorbed by the plant, but now we know that the food of plants, using the word in the well-defined sense of animal physiology, is not these substances, but the photosynthate and its derivatives. Doubtless the old usage is too firmly fixed to be changeable, but the real condition should upon all occasions be made plain. Some, though an insufficient, distinction can be made by terming the former raw food and the latter elaborated food.

LITERATURE OF CONVERSION. Upon this subject the most important work, in addition to those of PFEFFER and of JOST, is CZAPEK'S "Biochemie der Pflanzen" (Jena, Gustav Fischer, 1905), an exhaustive work upon every phase of this subject. There is an important article upon the reserve substances of plants by GREEN in Science Progress, **2**, 1894–95, 109, and **3**, 68, 476. Upon starch in particular there is the important work of MEYER, "Untersuchungen über die Stärkekörner" (Jena, Gustav Fischer, 1895), and in this country the subject has been studied especially by KRAEMER, Botanical Gazette, **34**, 1902, 341, and **40**, 1905, 305. Upon enzymes and their action, the most important work is GREEN'S "The Soluble Ferments and Fermentation" (Cambridge, 1901).

5. RESPIRATION (ENERGESIS) AND FERMENTATION.

We have now traced the formation of the basal organic substances and their transformation, or conversion, into the principal of the multitudinous special materials displayed by plants. But these are not immortal, and sooner or later they vanish. We must now trace their fate, which in general lies in one of three directions. *First* they are absorbed by animals, or by parasitic plants, of whose tissues they become a part. *Second* they decay by chemical and physical methods to be noted below. *Third* they disappear by an important process with which the student has probably already come into contact under an earlier suggested experiment (page 95). That experiment implied a loss of weight in growing tissues when photosynthesis is not in progress; and it is very easy, by experiments along the line of that suggested, or by others of like sort which the student may readily devise for himself, to prove that, excluding photosynthesis or other addition of substance, this loss of weight is universal in all living organisms where work of any kind, including even the bare maintenance of life, is in progress. Since, however, as the principle of the conservation of matter teaches, the vanishing material cannot be obliterated, its only possible fate would appear to be its escape in the form of a gas. Such a possibility entails far-reaching consequences, and hence calls for experimental study, thus presenting the problem:

Is any gas released by working tissues of plants, and if so, what is its identity?

This may be determined very readily by a test of the gas in a chamber in which working tissues have been kept for some time.

EXPERIMENT. Into the chamber of a respiroscope insert germinating oats or barley in suitable quantity, and stand the open end in a small dish of mercury; give favorable conditions for germination in darkness for two days, then apply tests for the gases present, beginning as usual with that for carbon dioxide (page 98).

PRECAUTIONS. While the seeds will germinate in the chamber if well soaked, it is practically best to germinate them first, in a saucer germinator, to some 5–10 mm. radicle length, for thus their progress may better be watched, their growth is more active, and bad ones may be excluded. The best temperature for growth is about 28° for these seeds, though the process here under study is more active at a higher temperature. A good proportion of seeds to chamber capacity is about one oat or barley grain to each 10 cc. capacity, though more seeds will give quicker results. It is of some advantage, though for this purely qualitative experiment not necessary, to start the experiment with the mercury some distance up the tube, for thus an expansion, as well as a contraction, of the contained gas may be shown. This may be effected by tipping the tube at the surface of the mercury, or, better, by sucking through an inserted fine rubber tube.

RESPIROSCOPES. These may be of any form offering a gas-tight chamber for the working tissues in continuity with a tube in which a visible gas test may be applied. A good form is described by DETMER, 261, and a very convenient form is the normal respirometer later described. A number of adapted forms, all made up from common articles of the laboratory, are shown by the accompanying figure (Fig. 30). In all cases the open end

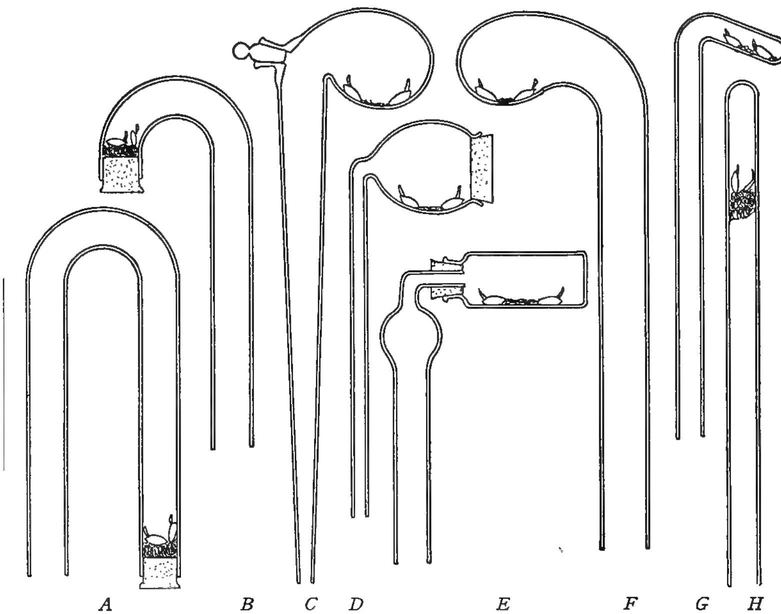


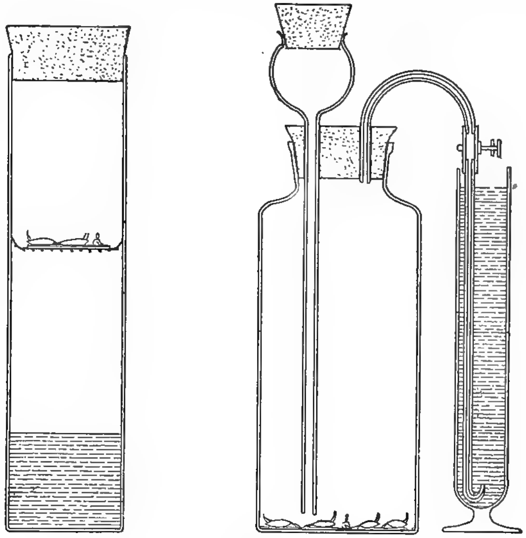
FIG. 30.—VARIOUS FORMS OF RESPIROSCOPES; ×⅛.
Explanation in text.

stands in a small dish of mercury (or other chosen reagent), and moisture is supplied the seeds by wet wool or sphagnum, or by a few added drops of water. The form *A*, made from an ordinary U tube (as described in Botanical Gazette, 27, 1899, 263) and used with the open arm in a tall bottle of mercury, is perhaps the most practicable for general work. In *H* the seeds must be supported upon a wad of loose glass wool or wire netting. In *F*, *G*, *H* the seeds must be inserted through the tube, which must then be dried by a mop if potash is to be used, or this will diffuse up the lines of moisture and kill the seeds. Another form in which chamber and tube are detachable by a ground-glass joint is described by RICHARDS in Torreya, 1, 1901, 28.

To these tubes the carbon-dioxide test may be applied either by the reagent tubes (page 105), or by introducing, through a curved pipette, a few drops of caustic-potash solution, or by inserting a small lump of solid caustic potash. For make-shift purposes the mercury may be replaced by water, whose absorption of carbon dioxide is not too great for its use in roughly qualitative experimenting.

DEMONSTRATION METHODS. Since the test for carbon dioxide is always the first to be applied, it is convenient and effective to apply this absorbent at the start; for thus the gradual progress of the gas release may be watched, while there is also this additional advantage, that the seeds grow somewhat better if the carbon dioxide is removed as formed. The caustic potash may be introduced over the mercury, or in solution may replace it, its absorption from the atmosphere not mattering, especially if the solution is in considerable bulk and small surface. This method, however, involves a logical error, which is commonly overlooked in this kind of experimenting, *viz.*, the rise of the carbon-dioxide absorbent does not of itself prove that any carbon dioxide has been formed, because the same result would follow if gas were absorbed by the seeds without giving off any, *i.e.*, if the seeds absorbed the oxygen in the tube without giving off any carbon dioxide. Hence it is necessary to add another tube similar in all ways except that it contains mercury, the approximately constant level of which shows that gas is released as well as absorbed, the two together proving that this released gas can be only carbon dioxide. Another method, a modification of the preceding, consists in placing a vial of caustic potash with the seeds in the chamber (which may be a flask or bottle), and supplying mercury to the open tube (compare DARWIN and ACTON, 2, and MACDOUGAL, 253). This saves the exposure of the caustic potash to the action of the air outside, but has the demerit that the weight of the mercury prevents a fully effective demonstration of the absorption.

For elementary and lecture purposes the demonstration is very effective with either of the arrangements of Fig. 31. *A* is a cylindrical bottle holding a quantity of clear lime-water, over which is suspended a diaphragm of wire netting supporting wet filter-paper on which are germinating seeds. When tightly stoppered and placed under good conditions for growth, the gradual whitening of the lime-water is striking, especially if it is well shaken at times, as this arrangement permits, and if it be tried beside a control lacking the seeds. Or the lime-water may be in a vial surrounded by the seeds. *B*, especially good for lecture demonstration, is a wide bottle, tightly closed by stoppers and clamp, on whose bottom is wet filter-paper containing germinating seeds. If at the end of two or three days water be poured down the thistle-tube, the gas of the jar can be driven through the tube, now unclamped (and preferably having a capillary point ensuring small bubbles and better absorption), into the bottom of a tall vessel of clear lime-water, an arrangement more effective if tried beside a control lacking the seeds. Another very effective lecture demonstration is afforded by use of the normal respirometer later described (page 129); after two or three days' growth of the seedlings therein, the rubber tube, first clamped near the measuring-tube (to keep clean the mercury in the reservoir tube), is cut (by scissors) under



A *B*

FIG. 31.—DEMONSTRATION RESPIROSCOPES; ×⅛.
Explanation in text.

the surface of a solution of strong caustic potash, when the mercury will run out and be replaced by the potash, which will rise in a striking manner as it absorbs the carbon dioxide.

ERRONEOUS EXPERIMENTS. Several errors or fallacies are current in connection with experimentation upon this subject. One of these has already been noted, *viz.*, that the rise of a carbon dioxide absorbing solution in a respiroscope tube shows that carbon dioxide is released by the seeds. Another of a different sort is the assumption that mercury rising in a tube as carbon dioxide is absorbed by caustic potash in the chamber, marks the extent of the absorption. In fact the mercury is so heavy that the air in the vessel is rarified, the more so the higher it rises. Hence the absorption is much greater than the mercury indicates, though by an amount easily calculated (see correction table in Part III). The same objection applies of course to caustic potash in the open tube, but to a degree so much less as to be negligible in elementary work, though attention should be called to it. But a more serious fallacy occurs in connection with the familiar make-shift experiment in which a candle or other flame lowered into a vessel containing seeds which have been germinating for some hours, becomes extinguished. By some this is taken as evidence that carbon dioxide has been formed, by others that oxygen has been removed, and by others that both of these things have happened. In fact it may be due to either of these things or to the formation of some other non-combustion-supporting gas, and taken alone, unsupplemented by other evidence, it is wholly illogical and inconclusive. Some loose or unfortunate ways of approaching the teaching of Respiration are discussed by SHAW in Science, 25, 1907, 627.

The release of carbon dioxide in conjunction with loss of weight shown by this experiment at once recalls the reciprocal phenomenon already studied in photosynthesis, and this in turn must raise the query whether the parallelism extends farther and includes an absorption of oxygen. Hence we have to determine:

Is oxygen absorbed by the working tissues of plants?

This may readily be settled by a test of the gas remaining in a chamber after the carbon dioxide formed by working tissues has been removed.

EXPERIMENT. After the application of the test for carbon dioxide to the respiroscope tubes of the preceding experiment, test the same for the presence of oxygen; this may most conveniently be done by use of the reagent tubes.

DEMONSTRATION METHODS. The disappearance, whole or partial, of the oxygen may most effectively be demonstrated by using the normal respirometer later described (page 129). A special arrangement for the purpose is described by DETMER in his "Kleines Praktikum," 139. The absorption of oxygen is, however, usually demonstrated indirectly by showing that all activity (growth, etc.) stops when none of that gas is available. Oxygen may be excluded by any one of several methods, controls being needed in all cases, as follows. (*a*) Germinating seeds of measured radicle length are placed on wet filter-paper in the bottom of a wide bottle having a very tight stopper pierced by a slender glass tube; for this a wide form of chemical wash-bottle is good, or a calcium-chloride tube may be found to nearly fit the neck of a bottle or conical flask where it may be made tight with sealing-wax. The air is then exhausted to a vacuum, and, while the exhaust is on, the tube is sealed in a Bunsen flame. After some days of good growth conditions, the bottle is reopened (by breaking the sealed tip) and the radicles are remeasured. The tightness of the joints can be assured by added sealing-wax, and a small mercury manometer should be inserted to demonstrate the vacuum. (*b*) An ingenious method of securing the exposure of seeds to a vacuum, without the use of an air-pump, has been described by L. MURBACH in School Science, 1, 1901, 25, and his method, somewhat modified, is as follows: Treat each of two glass tubes of about 4–5 mm. bore and 15 cm. length as follows: seal one end and push to this end two germinated Oats or Barley grains held in place by a spring-coil of wire; fill the tube one-third full of water previously boiled (to expel air) and cooled, and then draw out the open end in the flame to a slender point; holding the tube slightly inclined from the vertical, boil the water near its surface, which will not, because of the low heat-conductivity of water, injure the seeds; whilst the water is actively boiling, thus replacing all air in the tube by steam, quickly drop the point of the tube into the flame and seal it. When cool the condensation of the steam will leave a vacuum in the tube, the perfection of which may be tested in part by the "hammering" of the water when the tube is shaken lengthwise, and in part by the rise of water when, at the close of the experiment, the sealed point is broken under water (by pincers). In one of these tubes, the control, the point should now be broken, air readmitted, and the tube resealed; then both should be stood side by side (Fig. 32), seeds upwards under good growth conditions, and the comparative behaviors of the two sets watched. (*c*) Tubes, containing seeds, similar to those just described could be exhausted of air by an air-pump, and then sealed by the Bunsen flame. (*d*) The seeds may be introduced through a column of mercury in a tube over 76 cm. long, thus coming to lie in a Torricellian vacuum (compare DETMER, 275). (*e*) The air may readily be displaced by hydrogen from a bottle containing germinating seeds, either by simple buoyant displacement, or by expulsion of water over the pneumatic trough. (*f*) The seeds may be placed in the chamber of a respiroscope, the open end of which is placed in pyrogallate of potash; this will rapidly absorb the oxygen from the instrument, rising of course to take its place.

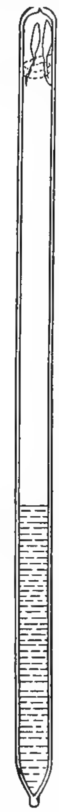


FIG. 32.—VACUUM TUBE CONTAINING GERMINATED SEEDS AND WATER; ×½.

The absorption of oxygen with release of carbon dioxide, shown by the foregoing experiments, recalls the similar phenomena in animals, where they are known to be associated with the vitally important process called *Respiration*. This resemblance, the student will find on further inquiry, is not one of accident, but of relationship, and the process is identical in the two kingdoms.

The foregoing study of the process has been purely qualitative, but we must now consider it quantitatively, not only because of the clearer definition and possible extension of knowledge such a treatment will give, but also in order to follow farther the suggestive parallelism with photosynthesis. Accordingly we face this problem:

What quantitative relationships exist between the gases exchanged in respiration?

This may be determined, of course, by accurate quantitative analysis of the air which, for an interval, has surrounded germinating seeds or other working tissues.

EXPERIMENT. Germinate some good oats or barley grains until the roots are visible (2–4 mm. long). Select about ten of these and determine their volume (by immersion in water in a small measuring-glass); place them with some moisture in the chamber of a respirometer, the measuring-tube of which contains a solution of caustic potash; give favorable conditions for growth and observe the rise of the solution; as soon as this has reached its limit, apply the test for oxygen and compare the volumes concerned.

PRECAUTIONS. The experiment must not be allowed to continue after the oxygen is absorbed, for then decay gases are released, to the detriment of the result. Of course the usual corrections for temperature, etc., must be applied, and care must be taken that the caustic potash cannot diffuse up streams of moisture to the seeds.

RESPIROMETERS. The various respiroscopes already described may also serve as respirometers if properly used, *viz.*, with the mercury level started some distance up the tubes, with allowance for volume occupied by the seeds and moisture, with proper corrections for temperature, etc., and with an accurate measurement of the volumes occupied by the absorbing liquids used in the tests. But of respirometric methods proper there are several. Thus the living tissues may be kept for a time in a closed chamber with a carbon-dioxide-absorbing substance, whose carbon-dioxide content is later determined, as in STICH'S apparatus (DETMER, 278); or a sample of the gas may be withdrawn for analysis by methods which RICHARDS has especially refined (Annals of Botany, 10, 1896, 531). RICHARDS has also described a suitable small chamber for seeds (Torreya, 3, 1903, 113). The commonly used method for exact work, which measures, however, only the carbon dioxide released, is that of SACHS (described in DETMER, 259, and in DARWIN and ACTON, 3), in which air freed from carbon dioxide is drawn by an aspirator over germinating seeds into tubes of baryta water, the amount of the carbon dioxide being subsequently determined by titration. This method has been improved by others, notably by BLACKMAN, whose apparatus is the most precise for this purpose yet constructed (Annals of Botany, 9, 1895, 161). The PETTENKOFER baryta tubes, much used in the analysis of the gas, are supplied by ARTHUR in his apparatus (Botanical Gazette, 22, 1896, 468). A great simplification of the apparatus for demonstration purposes is described by Reed in the Journal of Applied Microscopy, 5, 1902, 1891. Yet another form and method, one having the great educational advantage that every stage in the experimenting is visible to the eye, while at the same time the apparatus is simple, portable, and convenient of manipulation, is supplied by my new demonstration respirometer, described in the Botanical Gazette, 43, 1907, 274. It is supplied among my normal apparatus (page 46) and is constructed as follows:

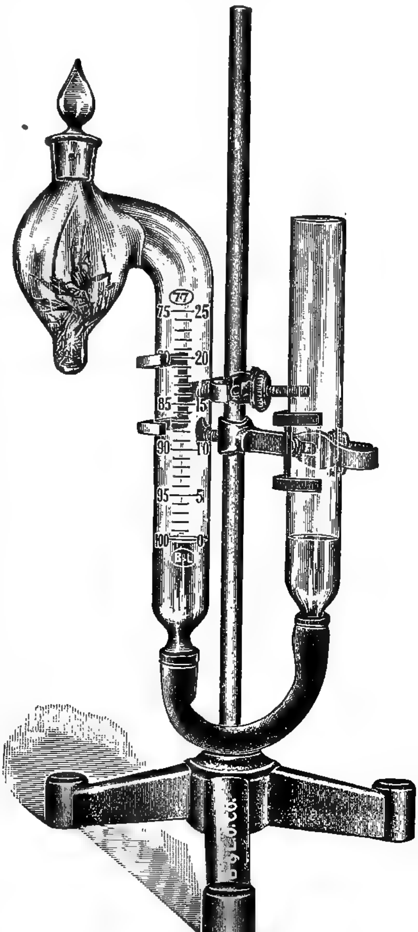


Fig. 33.—Respirometer; $\times\frac{2}{5}$.
Explanation in text.

The instrument (Fig. 33) consists of three parts. First is the oval chamber for the seeds, with a water-bulb at the bottom and a ground stopper having an air-opening matching with one in the neck. Second is the measuring-cylinder in open communication with the chamber, graduated from 75 cc. to 100 cc. of the combined capacity of itself and chamber, though the 75-cc. mark is actually placed at 77 cc. of the capacity. Third, and communicating with the preceding through a slender rubber tube, is the reservoir cylinder, ungraduated but with index marks 25 cc. apart. Both tubes are supported vertically by any convenient laboratory clamps which permit the reservoir tube to be slipped up and down. For demonstration purposes it is best to select seeds in which the oxygen absorbed and the carbon dioxide released are as nearly as possible equal in volume, *e.g.*, Oats. Ten of these of average size are soaked, or, better, are selected from a lot which have been started in a germinator until the roots are about 5 mm. long; they are then placed in the chamber, root ends down, just above the bulb, where they will stick if previously wetted. These occupy approximately 1 cc. of volume, and if now 1 cc. of water be placed in the bulb, there will be 2 cc. of these materials and 100 cc. of air, the composition of which is of course known, above the zero mark. Where greater accuracy is desired it may be attained by first dropping the ten seeds into a proper measuring-glass, then filling this with water to the 2-cc. mark, and finally placing both seeds and water in the chamber. The index liquid to be used is now poured through the reservoir tube until it stands level at the 100-cc. mark of the graduated tube and at the upper index mark of the reservoir (or the lower when mercury is used). The stopper, properly lubricated, is inserted with its air-opening matching that of the neck, and is then twisted, thus sealing the chamber without any compression of air. The apparatus is now shielded from light and placed under favorable conditions for growth. After the seeds have been growing for some three or four days, an analysis of the gas is made by the reagent-tube method described on another page (page 105). The reservoir and rubber tube are slipped off under water, allowing the mercury to run out, and are then used as the reagent tube, the reservoir being stoppered for the purpose. Or, perhaps more simply, the determination of the carbon dioxide may be made by removing the rubber tube from the measuring-cylinder under a solution of caustic potash, as described on page 125, while the reagent tube may be applied for oxygen in the usual way. The usual corrections must of course be made. The gas-pressure inside at the time of reading is equalized with the atmospheric pressure by sliding the reservoir tube up or down until the levels inside and out are the same, and these levels are to be equalized even in case the analysis is effected in another manner. For very exact work it would be necessary to take account of the barometric pressures, but the slight error from this source is negligible in demonstration. The temperature must either be made the same at the start of the experiment and the final reading, or else, as is readily possible, the change of volume due thereto must be calculated. Vapor-tension should also be considered in exact work, but it is negligible in demonstration. The size of the tubes is such as to eliminate all error of capillarity. After each use the instrument should be thoroughly washed clear of potash. It may seem an objection to a closed chamber respirometer, in comparison with the open types, that the oxygen is obtained under constantly increasing difficulties. But as STICH'S results (JOST, 202) and my own experience with this instrument show, this difficulty does not affect appreciably the absorption until near the limit, and then affects only the rate.

This quantitative study of the gases concerned in respiration suggests an extension of the inquiry to the loss of substance by the seed in comparison with the amount of carbon dioxide formed. For an exact study of this subject the experimental difficulties are great, and, besides, our knowledge of the precise substances used is very deficient. Nevertheless results of some value may be obtained by simple methods after the following:

Suggested Experiment. Prepare two sets, each of 10 seeds, of Barley or Oats, and weigh to a milligram; soak one set some 10 hours or more and place it in the chamber of a respirometer (not first germinating them elsewhere, which would entail some unmeasured loss of carbon dioxide), and supply caustic potash in the measuring-tube. Supply favorable germination conditions until the caustic potash has risen to near its limit, then determine exactly the volume of the carbon dioxide and the dry weight of the germinating seeds. In drying the latter also dry the other set of seeds, and use their percentage of moisture for determining the original dry weight of the germinated set, whence the actual loss of dry substance therein may be ascertained. Treating this substance, rather conventionally, as starch, and, using the formula $C_6H_{10}O_5+6O_2=6CO_2+5H_2O$, determine whether the starch used and the carbon dioxide formed agree when properly figured upon a molecular-weight basis.

The results of this experiment, despite its imperfections, will aid to confirm the supposition indicated by the earlier experiments, that respiration in these seeds exhibits phenomena seemingly reciprocal to those of photosynthesis; and they seem further to indicate that there must exist an equation, the reciprocal of the photosynthetic equation, which has the form $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. This may be termed the *conventional respiratory equation*, and, among all the chemical expressions of organic nature, it is second in importance only to the photosynthetic equation. But the student must here be warned that this equation, while it expresses the end result of the process, is by no means true for the immediate steps, which are vastly more complex than the formula suggests.

In the foregoing experiments germinating seeds were used as representatives of working tissues chiefly because of their convenience of manipulation in conjunction with their conspicuous activity. Moreover these particular kinds were deliberately selected because they are known to exhibit the gas exchanges in the simplest and theoretically most perfect ratio, namely, that in which the volumes of the gases absorbed and released are equal. In other tissues, however, and especially in many other seeds, this simplest ratio by no means always holds (and indeed it is a question whether in the seeds above recommended the ratio is not simply accidentally correct), and the student should now make himself acquainted with this phase of the subject, in part through the literature and in part through aid of the following:

SUGGESTED EXPERIMENTS. With the respirometer determine the respiratory ratio of tissues other than seeds, *viz.*, (*a*) opening flower-buds, (*b*) growing terminal buds, (*c*) young growing leaves, (*d*) young roots, (*e*) some succulent shoots of Cacti or Euphorbias, (*f*) other available living tissues. The instrument must of course be kept in darkness, and all injury to the tissues must be minimized.

Again, with the respirometer determine the respiratory ratio in other kinds of seeds, such as Flax, Peas, Radish.

In using the respirometer for this purpose, it is obviously indispensable to provide for a possible movement of the index liquid in either direction; hence the reagent in the measuring-tube must be started at a point whence it may range down as well as up.

In this connection the student should inform himself upon our knowledge of *the meaning of the various respiratory ratios, and of the products of imperfect and of excessive oxidation, and with the possible ecological significance thereof.* Along with this subject he should also make certain that he understands clearly *the mutual relationships of respiration and photosynthesis in green tissues, their coexistence there, their relative amounts under different conditions of light and temperature, their possible utilization of one another's products, and the conditions both of light and of temperature under which they may exactly balance one another.*

There yet remains one other marked feature of photosynthesis for the reciprocal of which we should now seek in respiration, namely, the absorption or storage (conversion of kinetic to potential) of energy; and the question arises whether any energy is released (converted from potential to kinetic) in respiration. Familiar facts connected with the process in the animal kingdom (*viz.*, the greater activity of respiration, as shown by the breathing, accompanying greater exertion) imply that this is so, but it is difficult to test directly in plants. There is, however, one fact about the transformations of energy in general which may be utilized for a test of this question, namely, no matter in what form energy is released, some of it always appears as heat. Our inquiry then resolves itself into this:

Is any heat released in the respiration of plants?

This may readily be determined by accurate thermometric observations of respiring, in comparison with similar, but non-respiring, tissues.

EXPERIMENT. Fill two non-conducting chambers of 50 to 100 cc. capacity with, respectively, actively germinating Peas (or young opening flower-buds), and the same freshly killed by hot water and cooled (in water containing 5 per cent of formalin, to prevent fermentation). Provide a carbon-dioxide-absorbing receptacle in communication with the chamber (thus promoting growth and respiration), and insert the bulbs of two thermometers, the most accurate and sensitive available, into the middle of the seeds; place under conditions favorable for respiration (say 28°–30°), and compare the thermometers at intervals during two or three days.

NON-CONDUCTING CHAMBERS. These are of all degrees of perfection, from the very perfect calorimeters used by various investigators in researches upon this subject, down to make-shift devices of small worth. A form commonly used for demonstration purposes is a glass funnel lined with perforated filter-paper, set over a dish of caustic potash, the whole covered by a bell jar (compare DETMER, 288). *Or* a tumbler may be used with the seeds resting upon a wire netting over the caustic potash in a flat dish on the bottom. *Or* inverted small flower-pots, surrounded by felt paper and resting on wire netting over a stick of caustic potash, may be used, as recommended in the first edition of this book. *Or* small beakers, one inside another with cotton between, closed by a cork perforated for a thermometer, and containing a wire netting over a stick of potash, are good. Far better, however, than any of these arrangements, and one giving admirable results, either for quantitative studies of some accuracy or for demonstration, is the arrangement shown in the accompanying figure (Fig. 34). It consists of two DEWAR bulbs made for liquid-air experiments, each of two concentric bulbs with a permanent vacuum between, and forming, therefore, very perfect non-conducting chambers. The caustic potash is introduced in a solid

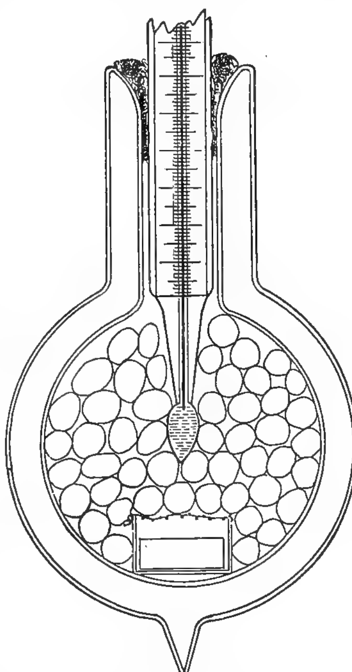


FIG. 34.—A SIMPLE CALORIMETER FOR STUDY OF THE HEAT RELATIONS OF RESPIRATION; $\times \frac{1}{2}$.

stick in a vial among the seeds, and the space between thermometer and neck is filled with cotton wool.

Instead of ordinary thermometers, a differential thermometer, with the two bulbs immersed in the two sets of tissues, is said to serve well for qualitative demonstration purposes (compare MacDougal, 263, and Darwin and Acton, 12).

The student should now enlarge his knowledge of this subject of heat release, testing it for himself, if possible, in the notable case of the opening flower of Aroids. At this point, also, he may best consider the general subject of the *comparative activity of respiration in various plants, inclusive of Fungi and Bacteria, and a comparison of their respiratory activity with that displayed by animals.* Here also should be considered other possible sources of energy available to plants.

The amount of energy released by the oxidation of a given amount of carbon, from any given compound, to carbon dioxide, is a perfectly definite and known quantity, and is expressed in terms of heat in calories (*i.e.*, the heat necessary to raise 1 g. of water 1° C.). The student should now ascertain the number of calories developed by the respiration of definite quantities (grams) of starch and of glucose, and he will do well to determine in these terms the energy released by plants in his experiments described earlier on page 131. He should also compare the respiration energy of these substances with the combustion energy of coal.

The release of energy in respiration is the most important subject with which the student has come into contact, or can come into contact, during his entire course. It is the central and crucial fact in respiration, the end and aim of the process, that to which all the phenomena of gas exchange, etc., are merely incidental. Consequently the student should now make certain of accurate knowledge as to its real physical nature. He should gain clear ideas as to *the forms of energy, their intertransformations and their relations to motion, how the dissociation of carbon dioxide converts energy of motion into energy of position, how this energy of position can be preserved through all the combinations into which the carbon may enter (the photosynthate and* *its transformations clear to the proteid and to the chyle and tissues of animals) so long as it can be kept from oxidation, and how this energy may be transformed from energy of position to energy of motion at any desired point when oxygen is allowed to unite chemically with the carbon.* He will be aided by an understanding of the phenomena of carbon combustion, of the action of storage-batteries, and even by so crude a physical analogy as the storage of speech in the phonograph. It is only through an understanding of these processes that he will be able to appreciate the fundamental significance of respiration in the life of organisms.

The foregoing experiments have led more than once near to a consideration of the effects of external conditions upon respiration. For some of these the difficulties of experimental study are considerable. But for one of the most important, temperature, an experimental study is possible as follows:

SUGGESTED EXPERIMENT. In the chambers of a differential thermostat, an instrument described later under Growth, place simple and similar respirometers, each containing the same weight of respiring seeds and the same strength of caustic potash. Run the instrument with a range of temperature from 25°–35°, and observe the comparative respiration as measured by the rate of rise of the reagent.

OPTIMUM TEMPERATURE FOR RESPIRATION. It is agreed by all observers that the rate of respiration increases with the rise of temperature to near the death point, the optimum thus lying very close to the maximum. Hence in theory, where respiration alone is under study, the higher temperatures give quickest results. But as the higher temperatures influence other functions of the plant unfavorably, it is in practice better to keep the temperature lower, and the practical optimum may be taken as 28°.

Closely correlated with respiration is the consideration of the free access of oxygen and removal of carbon dioxide, a subject which will be considered later under Absorption.

Among the various respiratory ratios shown by different seeds earlier studied, the student will have met with some (*e.g.*, peas and other legumes) which show a release of carbon dioxide out of all proportion to the amount of oxygen absorbed, implying that oxygen must in those cases be supplied from some source other than the air in the instrument. This at once raises the question whether, in the best marked of such cases, the carbon dioxide can be released if no oxygen at all is present, which presents this problem:

Can seeds of abnormally high respiratory ratio release carbon dioxide without presence of any oxygen?

This may readily be determined by placing the seeds in question under conditions favorable for respiration, except that all oxygen is excluded.

EXPERIMENT. Fill the tube, and half fill the reservoir, of an anaerobic culture vessel with clean mercury; take two or three fully soaked small Peas, remove their seed-coats (so that air may not be enclosed), plunge them (with forceps) under the mercury, and shake them free of air, then release them under the tube, when they will rise to its top and lie completely enclosed in the mercury. Give favorable conditions for respiration, and when gas formation has ceased, or nearly (after two or three days), apply the carbon-dioxide test by slipping a small piece of solid caustic potash into the tube.

ANAEROBIC CULTURE VESSELS. These may be of much elaboration for special studies (compare DETMER, 264), or may be much simpler and still efficient for our present use. They may consist of chambers in which the seeds lie in pure hydrogen gas (later analyzed for the carbon dioxide), or a Torricellian vacuum (DETMER, 275), or some form of "fermentation tube," of which the SMITH form, commonly used in this country, has rather too small a neck for the insertion of the potash, an objection which does not apply to the KÜHNE form (figured by DETMER, 263) But equally efficient, while readily adapted from common appliances, is the arrangement figured herewith (Fig. 35), consisting of a small test-tube supported inverted over a small glass mortar or other vessel containing mercury. If it were desired to make the study quantitative, a graduated test tube could be used, and the seeds could be withdrawn by very fine wires previously attached to them and extending through the mercury.

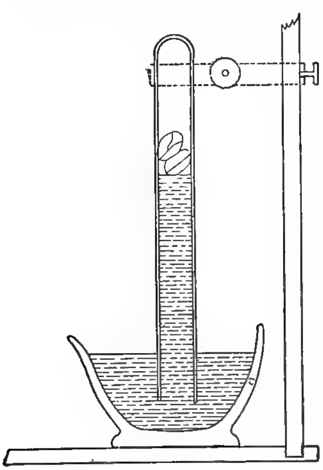


FIG. 35. — ANAEROBIC CULTURE VESSEL, USING MERCURY; ×½.

The formation of carbon dioxide anaerobically, that is, in absence of free oxygen, shown by this experiment, suggests an inquiry into the other products which may be formed during the process. Their determination offers great experimental difficulties, and hence the student must ascertain this point from his accessible literature. The results of his study will no doubt recall to the student another very conspicuous case in which, without free oxygen, carbon dioxide and alcohol are formed by actively working tissues, namely, in yeast fermentation. This process, partly because of its connection with our present subject and partly because of its immense economic importance, should be thoroughly understood by the student. As a basis for such understanding he should personally observe for himself:

What are the exact products of yeast fermentation?

This may be determined by inducing fermentation under conditions such that the products may be collected and submitted to analysis.

Experiment. In an Erlenmeyer, or conical, flask of about 200 cc. capacity, place about 100 cc. of a fermentable solution, and add thereto a half cake of compressed yeast (such as Fleischmann's, weighing about 12 grams); close the flask with a tight rubber stopper through which passes a thermometer and a glass tube bent as in the accompanying figure (Fig. 36); carry the free end of the latter into a dish of water over which is suspended an inverted and water-filled test-tube. Allow the gas from the flask to escape until all the air originally present must have been expelled, then allow the gas to rise into the test-tube until full, after which apply the test for carbon dioxide (insertion of a lump of caustic potash). Some hours later, after all gas evolution has ceased, examine the liquid in the flask, noting its smell, etc. Then proceed to distill it at a temperature below boiling. Examine the distillate and apply to it a test for alcohol.

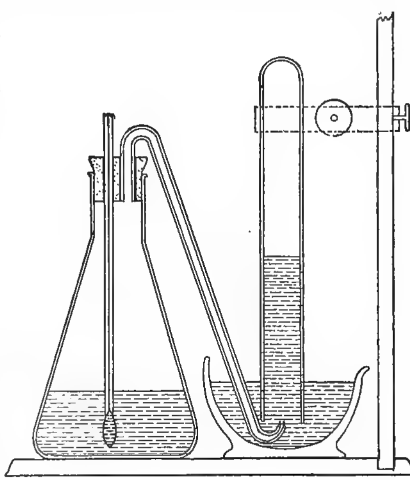


Fig. 36.—Arrangement for the study of fermentation; ×⅛.

Precautions. It is best to have the flask of a capacity at least double that of the liquid used, as otherwise the frothing during fermentation will carry some of the liquid over into the tubes. This, however, can be prevented by a plug of glass wool in the neck of the flask, or even, it is said, by a piece of paraffin in the liquid. The temperature of greatest activity of the process is about 28°.

FERMENTABLE SOLUTIONS. Best of all these for yeast fermentation is PASTEUR'S, made as follows: 838 cc. of pure water, 150 g. pure grape-sugar, 10 g. ammonium tartrate, 2 g. potassium phosphate, .2 g. calcium phosphate, .2 g. magnesium sulphate. For greater practical convenience in use, the mineral constituents may be powdered together (in a mortar) and kept in a tightly stoppered bottle. An optimum quantity of compressed yeast for the fermentation is 1 g. to about 15 cc. of solution. Instead of the grape-sugar one may use cane-sugar which, while not itself fermentable, is readily inverted to fermentable glucose and dextrose by an enzyme in the yeast cake; the cane-sugar is considerably slower in fermentation than the grape-sugar. Another excellent solution is molasses (which contains about 35% of cane-sugar and about 32% of grape-sugar, with 18% of water and some miscellaneous substances) diluted to about 20%. No solution of over about 30% sugar should be used, since yeast is inhibited, apparently by osmotic pressure, from development in solutions of higher strength.

While the above solutions are best for studies in which the fermentation is to be prolonged, they are by no means necessary for demonstration purposes, since a solution of either grape- or cane-sugar to which yeast has been added will ferment actively for an hour or two without the mineral constituents.

While FLEISCHMANN'S compressed yeast cakes are the most convenient source of yeast, starting fermentation as they do almost immediately after addition to the solution, other forms of the yeast are available, including the cakes of dried yeast which, however, is much slower in beginning action.

DEMONSTRATION. Yeast fermentation can very readily be demonstrated to a class, and even as a lecture experiment. For this I have found the arrangement figured herewith (Fig. 37) very effective. The solution having been placed in the flask and the yeast added, the outlet tube, of the form shown in the figure, is dropped to near the bottom of a slender cylindrical tube containing clear lime-water, the whitening of which as the gas comes off (commencing almost immediately) is very effective. Then on another occasion, after the fermentation is complete, the liquid in the flask is distilled, which can best be done by gently heating the flask with the Bunsen burner to about 80° (alcohol boils at 78°), and sending the vapor through a glass still, the distillate being collected in the test-tube beneath. After the collection of some of the distillate, the iodoform test for alcohol may be applied. A convenient arrangement of the apparatus, which may all be supported permanently on one support-stand, is shown by the accompanying figure (Fig. 37).

For elementary or make-shift purposes the demonstration of the alcohol is too difficult, though an application of the iodoform test to the fermented solution yields a faint iodoform odor; but the proof that carbon dioxide is evolved is very easy along the line of the experiment above recommended.

Or a fermentation, or other anaerobic, chamber may be used. *Or* the simple arrangement of figure 36 may be used, the gas being sent into lime-water instead of water, where a double proof of its identity is possible.

TEST FOR ALCOHOL. The only available one is the iodoform test, with which the student should make himself familiar by application to known amounts of alcohol before using it in the foregoing experiments. To the solution suspected to contain alcohol add enough of a strong solution of iodine in potassium iodide to turn the solution a marked brown; then add enough strong solution of caustic potash to cause disappearance of the brown color, when, if alcohol is present, there will be precipitated a quantity of light-yellow crystals, beautifully six-sided under the microscope; and at the same time there is emitted a characteristic iodoform odor.

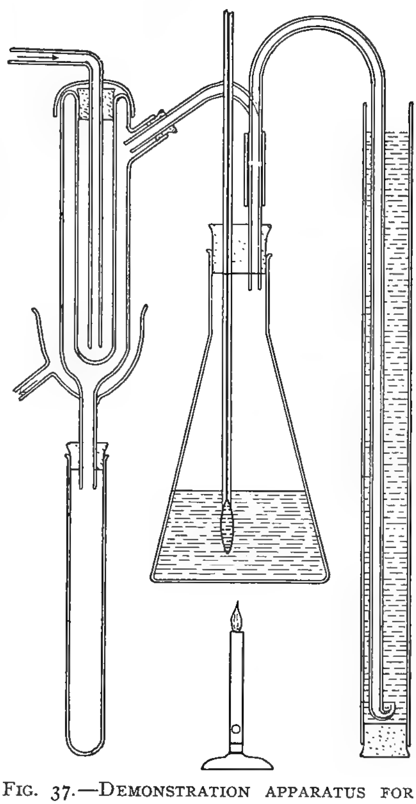


FIG. 37.—DEMONSTRATION APPARATUS FOR FERMENTATION; $\times\frac{1}{8}$.

On the left is the glass still, yielding a distillate in the test-tube below, in the center is the fermentation flask, and on the right is the tube of lime-water.

It will be of interest here for the student to renew his acquaintance with the structure and growth of the yeast plant, which he may very readily and profitably do by making a small culture in an open vessel from which he may draw samples, at intervals of a few minutes, for microscopic observation.

The association of respiration and fermentation must suggest the inquiry whether they show any resemblance in the very important feature of energy release. This may very easily be answered by testimony of good thermometers inserted respectively in the fermenting solution and in a control, *viz.*, a solution without the yeast, alongside.

If the student's course up to this point has been such as to inspire him with the genuine scientific spirit, he will be unwilling to leave this subject of fermentation until he has made his study of it quantitative, and he will wish to determine whether the carbon dioxide and alcohol formed equal the sugar used. An exact determination of this, especially for the alcohol, presents great difficulties, but for the carbon dioxide they are not insuperable, as may be found by use of the following:

SUGGESTED EXPERIMENT. Prepare the apparatus of figure 36, but without the thermometer and with mercury in place of water. Place in the flask one gram of grape-sugar with the suitable quantities of minerals, water, and yeast, and give favorable conditions for fermentation. Collect and measure the evolved gas, taking the carbon dioxide present in the flask at the close of the experiment to balance the air present at the beginning. Then using the equation $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$, determine the relation between the grape-sugar used and the carbon dioxide formed. Some error must be allowed for formation of a small percentage of other substances. The disappearance of the sugar may be tested by use of Fehling's solution.

The student should now extend his knowledge through the literature to include our present information upon *the energy relations of fermentation, the participation of enzymes therein, other forms of fermentation and their relations to enzymes, and the significance of the process to the organisms concerned.* Nor should he leave the subject without a clear knowledge of the leading facts in the economics of the process. And he should also make himself acquainted with the homologous processes involved in decay.

The student is now prepared to understand, and should make sure of his knowledge of *the interrelationships of aerobic and anaerobic respiration and fermentation, the chemical stages or steps therein, and the evidence as to whether respiration is at the expense of the living protoplasm itself, or of carbohydrates or other substances saturating the protoplasm.* Upon these important matters he will find the paper by BARNES cited below especially valuable. And, as always, he should express his arranged knowledge in a forcible exposition.

Respiration, with its need for access of oxygen and for removal of carbon dioxide, becomes the center of some important ecological features. Upon this subject the student, following the general method indicated below under photosynthesis (page 113), should now arrange and express his knowledge.

RESPIRATORY QUANTITIES. *As to the amount of carbon dioxide released,* for leaves the quantities determined by SACHS, by BROWN, and by BLACKMAN, in papers already cited under Photosynthesis, vary considerably, but approximate, respectively, to 30–40, 60, 80 cc. per square meter of leaf per hour at ordinary temperatures, of which we may take 60 cc., which is .12 grams, as the mean. Hence we may derive a conventional constant of .12 gm^2h for 20°. Respiration, however, rises very rapidly with temperature, approximating in leaves to 0 at 0°, rising to .12 gm^2h for 20°, to .30 gm^2h for 30°, to .65 gm^2h for 40°. It is to be noticed that BROWN claims a very much higher rate of respiration for greenhouse plants. As to seeds, certain figures given by JOST (193) would yield a conventional constant of 30 grams (15 liters) per kilogram of dry substance per hour at ordinary temperatures, that is, 30 *gkh*, and for buds about half that amount, or 15 *gkh*, while the corresponding amount for herbaceous leaves, which weigh about 30 g. per square meter of dry substance, would be 4 *gkh*. This *gkh* system is readily transformable to the *glh* system (page 22) by multiplying by .5 (carbon dioxide weighing approximately 2 grams per liter).

As to amount of energy released, it is shown by BROWN (Nature, 71, 1905, 522) that one gram of the conventional photosynthate yields on respiration with free oxygen 3760 calories (or more, according to others, *fide* PEIRCE, "Plant Physiology," 22), in round numbers 4000 calories. The combustion energy of one gram of carbon (charcoal) approximates 8000 calories, whence it follows that the respiration energy of the photosynthate is about half the combustion energy of an equal weight of the best fuel. BROWN has also shown that in a leaf of sunflower the respiration energy is equal to 349.2, in round numbers 350, calories per square meter per hour, which agrees well with an independent result obtained by calculation from the data above.

As to quantities of gases exchanged, this must be, conventionally at least, the reciprocal of the amounts in photosynthesis, earlier considered.

TERMINOLOGY AND CONCEPTIONS OF RESPIRATION. The term Respiration was early adopted from animal physiology, where it is still used loosely to signify not only the release of energy, but also the gas exchanges incidental thereto, and even the mechanical and forcible intaking and expulsion of air from the body, something not found at all in plants. To give greater precision to the central idea of the process, BARNES, in his paper cited below under Literature, has proposed the term *Energesis.* Since the old usage will not be displaced, the best we can do is to regulate its use, and we may well employ the terms for the future as follows: *Respiration*, an essential process in plants and animals whereby oxygen is brought into chemical union with carbon and hydrogen, with formation of carbon dioxide and water, resulting in *energesis*, or a release of energy utilizable immediately by the organism in its work, the mechanical movements of the gases being promoted in animals by *breathing*, or forcible muscular passage thereof in and out of the body.

It has of late been insisted by some of the younger physiologists of America that the processes of photosynthesis and respiration are not comparable reciprocally point by point as often taught by our current books. In some part the objection is well taken, but in much larger part it is simply a phase of the tendency to over-refinement of thought and expression apt to accompany too concentrated a specialization. Since all organic substance of both plants and animals starts from basal carbohydrate, which is formed with storage of energy from carbon dioxide and water, and since in the long run all organic substance, whether by respiration or decay, returns to carbon dioxide and water with release of the equivalent energy, it is quite plain that in the long run, that is, in the sums total, the two processes are strictly comparable. Moreover this is true even within much narrower limits, and it is even possible, as the instructive case of fermentation shows, that respiration in plants is a decomposition of carbohydrates saturating the protoplasm rather than of the protoplasm itself, the regular exchange of the gases being complicated by accessory chemical reactions, in part incidental and in part ecological. At all events, if safe-guarded by the knowledge that, of the photosynthate formed, only a part is soon used up in respiration (though in the end it all is), and that the steps in respiration are many and complicated while in photosynthesis they are few and direct, then the two processes are, even though somewhat conventionally, comparable.

Literature of Respiration. In addition to the general works of Pfeffer, Jost, and Verworn, there is much on this subject in the papers by Sachs, by Brown, and by Blackman, cited earlier under Literature of Photosynthesis. There is also a very valuable address by Barnes, "The Theory of Respiration," in the Botanical Gazette, **39**, 1905, 81 (and in Science, **21**, 1905, 241). On fermentation the basal work is Green's "The Soluble Ferments and Fermentation," Cambridge, 1901, while the most useful works upon yeast fermentation are Matthews' "Manual of Alcoholic Fermentation," London, 1902, and a very valuable lecture by van't Hoff in his "Physical Chemistry in the Service of the Sciences," Chicago, 1904. On the nature of the release of energy in the organism (animal, but applicable in principle to plants), there is a good article by Thurston in Science, **1**, 1895, 365.

6. ABSORPTION.

In the foregoing study of the formation, transformation, and deformation of the organic substances of the plant, we have more than once been brought into touch with problems involving the mode of movement of the substances. Obviously these are problems of physics, primarily concerned with the energy impelling the movements, and involving three phases: (*a*) First entrance of substances into the organism, or *absorption;* (*b*) removal from one part of the plant to another, or *transport;* and (*c*) removal from the plant, or *elimination.*

Of all substances absorbed by plants the most obvious and abundant is water, which, therefore, may best be considered first. The water-absorbing structures of the higher plants are the young roots with their hairs, of which the student should now review his knowledge (by aid of new microscopical studies upon germinator-grown material if necessary) until he understands fully, and can *diagram clearly, the structure and connections of tip, hairs, cortex, and vascular system, both as to walls and protoplasm,* and he should inform himself upon the composition of the cell sap in these various parts. The study will show plainly enough that the absorption of water is through solid walls which are without openings even when viewed under the highest powers of the microscope. Faced thus by a question of physics, the wise course for the student is to ask of that science whether any process is known whereby liquids are absorbed through imperforate membranes. And an answer is ready. Such absorption can occur through *osmosis,* which is a process involving a solution of some diffusible substance separated from the water by an imbibition membrane. With these matters the student must now make himself well acquainted, in part through study of the proper literature, but in part through personal contact with the phenomena, which he can best bring clearly before him in the form of this problem:

What are the actual phenomena, and the physical explanations of diffusion and imbibition?

EXPERIMENT. Fill with water an erect test-tube or equivalent, and stand it in a solid place of even temperature. Drop quickly to the bottom a lump of solid fuchsin, and observe the movement of the color, distinguishing convection and other movements from that of diffusion Also, replace the fuchsin by some colored solution, poured through a thistle-tube to the bottom.

EXPERIMENT. (*a*) Suspend a strip of membrane (page 146) and a strip of filter-paper each of the same measured dimensions, such as 5×2 cm, with one end in water, and observe the comparative rate of ascent of the water; then wholly immerse them for a time and measure any change of dimensions. (*b*) Prepare a smooth piece of soft wood, some $5 \times 5 \times .5$ cm., and measure, preferably with vernier calipers, its exact dimensions, cover one face with wet filter-paper until it warps, then note the force needed to flatten it, which will give some rough measure of its power of absorption; immerse it wholly in water until saturated and remeasure the dimensions; then dry it thoroughly and remeasure. (*c*) Select a hygroscopic awn of *Erodium*, *Avena*, or *Stipa*, and mount it vertically upon a cork by aid of sealing-wax; bring it into places of various humidities, from the dryest possible to saturation, and observe the resultant movements. (*d*) Mount a thin section of dry *Laminaria* stem (or cross-sections of coats of flaxseed) in strongest alcohol for the microscope, and, with the object under observation, draw in water to gradually replace the alcohol, and observe result.

In connection with these experiments the student should now make himself acquainted with *the physical nature of diffusion, especially with respect to the molecular state of the solute, the energy producing the diffusion movement, the diffusive capacity of the various common substances (salt, sugars, potassium nitrate), the distinction between crystalloids and colloids, the nature of imbibition by membranes with the molecular states and energy involved and its relation to capillarity, Nägeli's micellar theory of membrane structure and water movement therein, and the physical basis of hygroscopic movements of dry tissues.* Then he should proceed to the study of osmosis proper, which he may well approach thus:

What are the actual phenomena of osmosis?

EXPERIMENT. Into an osmoscope, preferably one with a cup membrane for rapid action, pour a suitable quantity of a strong solution of sugar, for which molasses is very convenient. Support the instrument upright with the membrane in pure water, and observe the result.

Also test the result when water is placed inside the osmoscope and the solution outside.

Also observe the result when two different strengths of the same solution are used.

OSMOSCOPES AND OSMOMETERS. An osmoscope consists essentially of an open tube over one end of which is tightly tied a membrane, the given solution being placed in the tube with the membrane immersed in water. Since, however, the visibility of the osmotic movement, as measured by rise of liquid in the tube, is directly proportional to the size of the membrane and inversely proportional to the size of the tube, it is desirable that the membrane be made as large as possible, either by greatly enlarging the bottom of the tube, or else by using the membrane in the form of a cup or bag. Several forms of good osmoscopes are illustrated in the accompanying figure, all adapted from ordinary laboratory materials. Upon the whole the most convenient is that shown by the letter *F*, using a Schleicher and Schuell diffusion shell of 16 mm. diameter (a suitable supporting glass ring for which is supplied with my normal apparatus, page 46). In all of these forms it is essential that the connection between membrane and glass be perfectly tight, though this is not at all difficult to accomplish if the membrane is first soaked in water (five to ten minutes) and is then tied tightly in place with strong well-waxed thread. In the construction of osmoscopes regard must be given to convenience of inserting the solution, which is readily

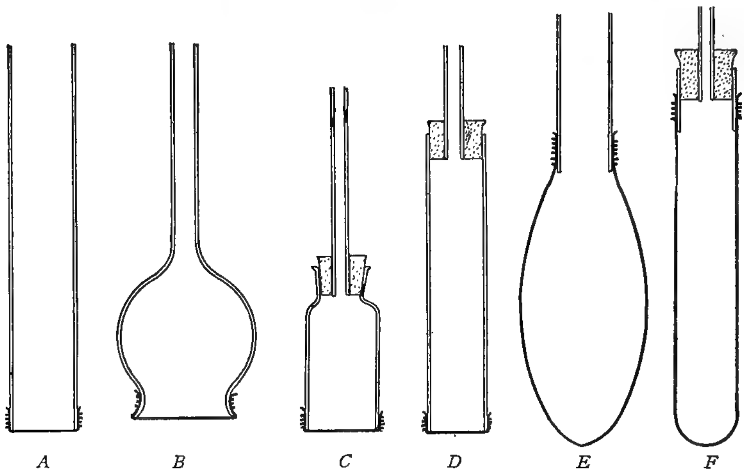


Fig. 38.—Various forms of adapted osmoscopes; $\times \frac{1}{2}$.
Double lines are glass, black lines are membranes, and dotted areas are stoppers.

effected in the stoppered forms, but may be accomplished, though with some trouble, in the thistle-tube form by pouring the liquid cautiously down a wire. A somewhat well-known make-shift instrument, more troublesome, however, to prepare, as I should suppose, than some of the above, is that using the membrane of an egg, described by Bergen in his elementary text-books.

An osmoscope of this kind may very readily be converted into an osmometer by using a graduated tube and a known quantity of solution. Further, it would be possible to make any of these forms autographic by use of a frictionless float and a recording wheel like those later described under Growth. Where an experiment is long continued, the molasses or other solution may be kept from fermentation by the addition of 2–3% of formalin, while evaporation may be checked by a covering of oil.

DEMONSTRATION METHODS. The form *F* of figure 38 is very effective, as the rise due to osmosis, molasses being used, progresses fast enough to yield a visible result within an hour. And the cup, when cleaned, may be dried and preserved attached to its ring, ready for future use. Far more effective, however, especially for lecture-table demonstration, is the arrangement originally described by MACDOUGAL (Journal of Applied Microscopy, 1, 1898, 56) and shown modified in figure 39. A piece of parchment-paper tubing (see section following), some 15 cm. long, is soaked a few minutes in water, then pleated and tied tightly together with waxed thread at one end, while the other end is tied tightly over a good stopper (preferably rubber) pierced by two holes. Molasses is poured through one of the holes until the cup is filled; then into one hole is inserted a stout tube of about 50 cm. length and 1 mm. bore (a smaller bore works badly because of the viscosity of the molasses), which is all the better if white-backed and of the magnifying form (pear-shaped in section). Into the other hole is inserted a thistle-tube or funnel with an interposed stop-cock. The cup is then supported upright in a vessel of water, which, for quickest results, should be lukewarm. Enough water is now poured into the funnel to raise the molasses into the tube, and the stop-cock is closed, when within a few minutes the liquid will begin to rise in the tube at a rate of several millimeters per minute, so that its progress can be seen by the class from a distance. As it nears the top the stop-cock is to be opened, when the liquid will drop back to the starting-point. Moreover, by removing the tube and funnel, the filled cup with stopper may be kept stored in a slender bottle of molasses, and is always ready for immediate use upon simply rinsing it with water. This simple arrangement really leaves little to be desired as an effective demonstration of osmosis. It could also be used to demonstrate pressure by sealing the tube at the top with sealing-wax, and calculating the pressure by BOYLE'S Law, though, owing to escape of the solution, only the existence of pressure and no approach to its true amount can thus be shown. A method of demonstrating pressure by use of a thistle-tube is described by E. A. BOGUE in Science, 13, 1901, 791.

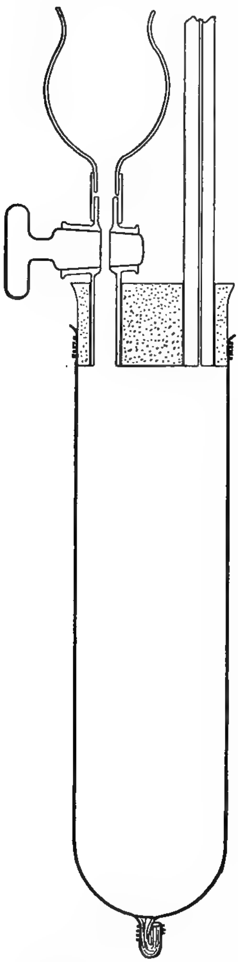


FIG. 39.—DEMONSTRATION OSMOSCOPE; $\times \frac{1}{2}$. Explanation in text.

MEMBRANES. Of these a number are available from both animal and plant sources. Thus there is *parchment* (animal skin), formerly much used by bookbinders and still obtainable from them; *animal bladder*, obtainable sometimes in the fresh state, and always in a cleaned and dry state from supply companies at a cost of about 10 cents each; *gut membranes*, in which sausages are placed, having the advantage that they may readily be used in the cylindrical form; *egg membrane* (lining the shell of an egg), which is difficult to remove uninjured by pulling off the shell, but which can be obtained to perfection by dissolving away the shell in hydrochloric acid of about half strength; and, finally, *parchment paper* (a tough paper so treated with sulphuric acid that the fibers are partially dissolved and allowed to melt together again without appreciable openings), obtainable at a very low cost from all supply companies, either in sheets or in cylinders of indefinite length and 40 mm. diameter. It is either with the animal bladder or with parchment paper that much of the earlier work upon osmosis (or dialysis) was done. Yet another kind, extremely useful for demonstration purposes, is now available in the *diffusion shells* (cylindrical round-bottomed cups) of SCHLEICHER and SCHUELL. They are of two sizes, both 10 cm. long, but of 16 mm. and 40 mm. diameter respectively, obtainable from supply companies at a cost of about 25 cents each. Another and very different kind of membrane is that formed by films of collodion, which may be made into cups or tubes, a substitute for the diffusion shells, by the method described by KARL KELLERMAN (in Journal of Applied Microscopy, 5, 1902, p. 2038). In synopsis the method is this: into a clean test-tube pour enough 3% collodion to coat the tube when it is inclined and rotated; when the collodion begins to dry, rest the test-tube inverted over a screen or blocks so the excess may drain off and the remainder may dry; let it stand for 5 minutes to an hour, then fill with water, when the tube will loosen and may be drawn out. Longer drying makes a tougher tube but an osmotically slower one, as does, of course, greater thickness or a thicker solution of collodion. Such tubes will keep for some time in water, though they become brittle in air. They form excellent, though very slow, osmotic membranes, and hence should be used as thin as practicable.

The student should now apply his knowledge, gained in connection with the immediately preceding experiments, to an interpretation of the facts of osmosis here observed, and should follow the subject in the literature until he understands what is known as to *the physical forces producing the entrance of the water against the increasing hydrostatic pressure, and what determines the limit of the rise.*

While the preceding experiment demonstrates an osmotic absorption of water under conditions somewhat resembling those in root-hairs, at the same time it shows one great difference, namely, the solution escapes through the membrane, something which does not occur in the case of the roots. Thus our experimental membrane is permeable to both liquids, while the roots are permeable to water but not sugar, in other words, are semi-permeable. In pursuance of this matter we again turn to physics and ask whether semi-permeable membranes are known, and we find that they are; so here arises this problem:

What are the osmotic phenomena where membranes are semi-permeable?

Experiment. Fill an upright test-tube or equivalent with a 5% solution of potassium ferrocyanide (using care, for it is poisonous), and into it quickly drop a small lump of copper chloride, which should sink to the bottom. Observe the growth of the resulting membrane, the physical conditions of which should be understood with a clearness permitting their diagrammatic representation. (The structure formed is sometimes called, from its discoverer, the Traube cell.)

Experiment. Prepare an osmoscope as for the preceding experiment, preferably the diffusion-shell form *F*. Immerse the well-soaked cup, attached to its tube, in a 3% solution of copper sulphate, and leave it to soak for some hours, preferably also exhausting the air while it is immersed. Then empty the cup, rinse it in distilled water, refill it to the glass with the potassium-ferrocyanide solution, replace it in the copper-sulphate solution, bring the two liquids to one level, and leave it twenty-four hours (to form the semi-permeable membrane). Then pour out the liquid and replace it by molasses or other selected solution; add, by a rubber-tube connection, a tube of the diameter of the glass ring, immerse the cup in water and observe the rise of the liquid until it stops; then test the water outside for the presence of sugar.

Semi-permeable Membranes. Only a few of these are known, including those formed of calcium phosphate (made as recommended by Detmer, 147, and by Darwin and Acton, 124), of gelatin (or mucilage), and of tannin (Pfeffer, 1, 106), but the best known and most useful is that of potassium ferrocyanide, made as indicated by the experiments above, after the following reaction: $K_4Fe(CN)_6 + 2CuSO_4 = Cu_2Fe(CN)_6 + 2K_2SO_4$. The membrane forms only when the contact of the substances is gradual, for if they are mixed in solution there results only a flocculent brown precipitate. The membrane unfortunately is very fragile, and hence for experimental purposes must be supported by some firm substance. For this purpose parchment serves well so long as no pressure develops, but as soon as it is stretched by pressure the delicate membranes break. Hence for all experiments involving pressure it is needful to support them upon an unyielding substance, for which porous porcelain, the finer in texture the better, is used. There is also an electrolytic method, somewhat too complicated for our present use, of forming the membranes in clay supports, as described by MORSE in his paper cited under Literature.

A method of demonstrating, by projection upon a screen, the formation of these membranes to an audience, is described by PFEFFER in his paper earlier cited (page 23, note).

The notable rise of the liquid against hydrostatic pressure in the preceding experiment implies an equivalent osmotic pressure within the solution, and the question arises as to how great this may be. The exact study of the subject is beset with experimental difficulties, but the student may follow it by aid of the following:

SUGGESTED EXPERIMENTS. Some idea, though crude and inadequate, of the osmotic pressure may be obtained by replacing the slender glass tube of *F* in figure 38 by a simple pressure-gauge; but as there is an escape from, as well as absorption into, the cup, it is plain that the full pressure cannot be measured except when the membrane is semi-permeable. The parchment membranes, however, are all too weak and yielding to serve as supports for such membranes (though they can easily be made to burst by the pressure), and for this purpose firm supports, such as clay cells, must be used. The best arrangement by far, where moderate pressures are concerned, is PFEFFER'S cell, an instrument which has become classical since the great physical importance of its results has been understood, though for stronger pressures the special modification of this used by MORSE is required. The student, if his time permits, should now prepare a PFEFFER'S cell. He will find great difficulties to overcome, but ample reward if he triumphs. It is described and figured in PFEFFER'S "Osmotische Untersuchungen" (Leipzig, 1877, 22), and the essential parts of the description, with the figure of his final arrangement (Fig. 40), is in HARPER'S "Scientific Memoirs," IV, 1899. Another figure, with a very valuable account of precautions necessary to success, is in GOODALE'S "Physiological Botany," 227. I have found that an excellent substitute for PFEFFER'S cups, which

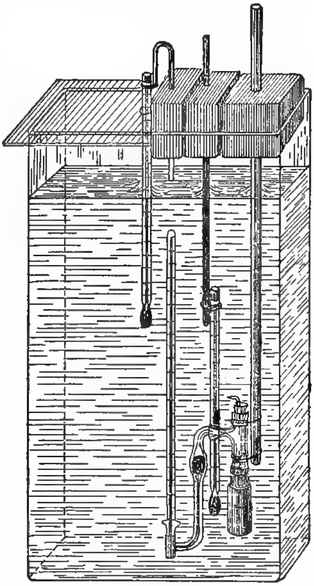


FIG. 40. — PFEFFER'S ARRANGEMENT FOR THE STUDY OF OSMOTIC PRESSURES; FIGURE REDUCED TO $\frac{3}{4}$.

(From PFEFFER'S "Osmotische Untersuchungen.")

are difficult to obtain, is a PASTEUR-CHAMBERLAND bougie filter, obtainable of all bacteriological supply companies at a cost of about 60 cents. I have also found that the gauge may be brought into communication therewith by use of a plumber's ground brass joint (thinly greased), one part of which is cemented by hard sealing-wax to the bougie and the other to the glass gauge, the various joints being made tight by glass sleeves and sealing-wax. Molasses, however, is not to be used in such a gauge, since its strength soon bursts the cup; and besides it is far preferable to work with the dilute solutions found in the plant.

The student should now inform himself as to our knowledge of *the amounts of osmotic pressure developed by different substances, both outside the plant and within, the relations which exist between osmotic and gas pressures, the relations of the pressures of different substances to the numbers of molecules involved and to the ionization of the substances, and the theories now held to account for the pressure.* And he should make sure that he understands the invaluable DE VRIES-PFEFFER table of osmotic data (PFEFFER, I, 146), excepting for the isosmotic coefficients, which are later to be studied.

So much for the physical and general aspects of the subject; we turn next to the direct observation of the osmotic phenomena of single living cells. Obviously these do not admit of direct osmometric measurements, but advantage may be taken of the fact that exosmotic is as easy as endosmotic movement, and an exosmotic movement may be induced by the use of strong solutions outside the cell. We may now bring the subject definitely before us in the form of a problem thus:

What phenomena are exhibited on the application to living cells of strongly osmotic solutions?

EXPERIMENT. Mount for the microscope, on one slide but under separate covers, streaming cells of *Nitella*, and apply to both, by drawing under the cover with filter-paper, a normal solution of cane-sugar, keeping one cell under observation the while. As soon as a marked effect (plasmolysis) is produced, replace the sugar in one by water, but leave the other for an hour; keep the preparations from drying out, and both observe and interpret the results.

EXPERIMENT. To other specimens apply, respectively, .5, .25, .24, .23, .22, .21, .20 normal cane-sugar, and observe the effect, leaving them for one hour. Which strength just produces a visible plasmolytic result?

EXPERIMENT. Repeat the preceding experiment, but using potassium nitrate in strengths .5, .25, .20, .19, .18, .17, .16, .15 normal; incidentally compare rapidity and completeness of plasmolysis caused by corresponding strengths of sugar and potassium nitrate, but especially observe which strength just produces plasmolysis, and therefore exactly equals the exosmotic power of the crucial strength of cane-sugar.

EXPERIMENT. Repeat the two preceding experiments with a colored cell-sap, preferably the now classical red epidermis of *Rhœo* (or *Tradescantia*) *discolor*, or else red Beet, or *Tradescantia zebrina.* Determine the solutions of cane-sugar and potassium nitrate which are isotonic (or isosmotic).

DEMONSTRATION METHODS. A method of projecting a plasmolyzing cell upon a screen so as to be visible to an audience is described by PFEFFER in his paper earlier cited (page 23, note).

NORMAL SOLUTIONS. In these studies percentage solutions (*i.e.*, those containing the percentage of the substance in grams dissolved in enough water to make up 100 grams) could of course be used, but, in view both of the nature of osmotic pressure and also of present usage in physics and chemistry, it is much better to employ equimolecular, commonly known as *normal*, solutions. There is some diversity of usage with regard to them, but the most logical, and that towards which usage seems to be tending, is this: a normal solution is one containing one *gram-molecule* (*viz.*, the molecular weight in grams) of the substance in enough water to make one liter of solution. In practice it is made by placing the proper weight of the substance (thus: of cane-sugar 342 grams, of potassium nitrate 101 grams) in a suitable measuring-flask with enough distilled water to dissolve it, and then adding enough distilled water, all at standard temperature, to make a total volume of one liter. Obviously solutions thus made contain, volume for volume, the same number of molecules of solute regardless of its chemical composition. Some students, however, especially formerly, simply dissolved a gram-molecule of the substance in one liter of water, which made the total volume different for different substances, and hence not strictly equimolecular, volume for volume. On the other hand some chemists prefer a somewhat different usage, and one of much less value for our present purposes, as follows: a normal solution is one containing one *gram equivalent* (*viz.*, the weight in grams which will react with a gram-molecule of a monovalent compound) of the substance in enough water to make one liter of solution. Thus of H_2SO_4, not 98 grams would be used, but 49; and therefore the gram-equivalent normal solution may contain either the same amount or $\frac{1}{2}$, $\frac{1}{3}$, or $\frac{1}{4}$ the amount of a gram-molecule normal solution. The two normal solutions may be distinguished respectively as *molecular normal* and *equivalent normal*, or, better, following ARRHENIUS, the former may be distinguished as N, while the latter are distinguished as $N\frac{1}{2}$, $N\frac{1}{3}$, etc., the two kinds being of course identical in monovalent substances, as well as in all neutral organic compounds. The normal solutions when diluted to lower strengths are designated as decinormal, etc., and written, N .1, etc. Others write them in fractional form thus: $n/8$, $n/16$, etc.

(Normal solutions are especially well discussed in LIVINGSTON'S "Rôle of Diffusion and Osmotic Pressure," cited in the Literature, and in ARRHENIUS' "Text-book of Electrochemistry," translated by J. MCCREA, 1902, 9, 10. The article by DANDENO in Botanical Gazette, 32, 1901, 229, may be noted, and KAHLENBERG'S reply in the same volume, 437.)

The student is now in contact with facts and phenomena not only of great interest in themselves, but important for the part they have played in the development of the physical chemistry of osmosis, and he should make himself acquainted with the classical work of DE VRIES thereon. He should make sure of an understanding of, and should clearly describe, (*a*) *the use of plasmolysis as a method of determining the comparative osmotic power of different substances;* (*b*) *the meaning of De Vries' isotonic* (*or isosmotic*) *coefficients, in their relations to the degrees of ionization of the respective substances;* (*c*) *the relation of these coefficients to the theoretical pressures of equimolecular solutions.* He should also take note, and may profitably repeat, DE VRIES' other method of determining these coefficients, *viz.*, by the elastic bending of strips of soft tissues.

OSMOTIC QUANTITIES. These are given with the most satisfactory fullness and clearness in the important DE VRIES-PFEFFER table in PFEFFER'S "Physiology," 1, 146 (reprinted by MACDOUGAL, 338). Osmotic pressures in the cells of the higher plants range generally from 5 to 11 atmospheres, rising to 21 in some special structures, but in the lower plants the pressures range very much higher, even to 160 atmospheres in some Fungi. We can therefore hardly express any conventional constant for osmotic pressure in plant cells. Cane-sugar gives a pressure of 22.7 atmospheres for a normal solution (*viz.*, a 34.2 solution), and the same pressure is yielded by normal solutions of all substances which are not ionized, with higher pressures for those which are. MORSE has actually measured over 34 atmospheres for cane-sugar. But much weaker strengths prevail in plants. The pressures given by the three substances most concerned in osmotic pressures in the plant, *viz.*, cane-sugar, grape-sugar, and potassium nitrate, are, respectively, .69, 1.25, and 3.50 atmospheres for a 1% solution, in the proportion to one another therefore of 1, 2, 5.

LITERATURE OF OSMOSIS. Osmosis, especially with respect to osmotic pressure, has attracted great attention in recent years from students of physical chemistry, with a correspondingly copious literature. The foundation works are two botanical investigations, the first by PFEFFER, who made the first actual measurements of osmotic pressures, with results contained in his "Osmotische Untersuchungen," Leipzig, 1877, and summarized in his "Plant Physiology," Volume **1**. The second paper is DE VRIES' "Eine Methode zur Analyse der Turgorkraft," in Jahrbücher für wissenschaftliche Botanik, **14**, 1884, 427, in which he gave a large number of pressures determined by comparison with definite standards, which were plant cells, and he showed a relation between osmotic pressure and equimolecular solutions. The later physical studies upon osmotic pressure, which have largely turned upon the correspondence between osmotic pressures and gas-pressures, are all admirably summarized in a work well-nigh ideal for the use of the student in this course, LIVINGSTON'S "Rôle of Diffusion and Osmotic Pressure in Plants," in the University of Chicago Decennial Publications, 1903. There is also a very important chapter by VAN'T HOFF in his "Physical Chemistry in the Service of the Sciences," in the same series, 1903, which sets forth very clearly his theory that osmotic pressures and gas-pressures are essentially identical in kind. This is consistent with the later measurements of pressures by MORSE and others, who used PFEFFER'S method improved in details, with results described in the American Chemical Journal, **34**, 1905, 1, and later. Other students have found some support for a surface-tension theory of osmotic pressure (Nature, **72**, 1905, 541). More recently the whole subject has been restudied by KAHLENBERG (Journal of Physical Chemistry, **10**, 1906, 141, and Philosophical Magazine, **9**, 1906, 214) to a conclusion strongly against the gas-pressure theory and in favor of the older explanation of an affinity between solute and solvent. But KAHLENBERG'S work has been criticized and must be confirmed (Nature, **74**, 1906; see Index).

The phenomena of plasmolysis already studied imply that it is the turgor of the osmotically tense cells which keeps the tissues firm and stiff, a matter of such importance as to deserve experimental study:

What effect is produced upon soft tissues by neutralizing the osmotic pressure inside the cells?

This end may readily be effected by immersing the tissue in a solution stronger than that inside the cells.

EXPERIMENT. Select a soft-textured leaf, one lacking any waterproofing bloom or wax, such as *Coleus*, Heliotrope, or *Primula*, and immerse it in a normal, or, for extremely rapid action, saturated, solution of salt or sugar, and observe and interpret the effect soon produced; then place it for a time in pure water.

DEMONSTRATION METHODS. The above method, when a saturated solution is used, yields a collapse of the leaf within 15 or 20 minutes, and hence may effectively be shown to a class, but the recovery requires an hour or more. A very striking lecture-table experiment to demonstrate the effect of osmotic pressure in giving turgescence, and hence mechanical rigidity, is the arrangement shown by the accompanying figure (Fig. 41). It is made from parchment-paper tubing tied at one end to a stoppered half vial and at the other to a glass stop-cock. It can be filled through the vial with molasses; and then the air may be allowed to escape, or the pressure may at any time be relieved, through the stop-cock, so that it hangs perfectly limp in a tape loop, as shown by the lower part of the figure. If, now, this be suspended in a glass dish of clear, lukewarm water, it will, well within an hour, become strongly turgescent and straighten stiffly out, as shown

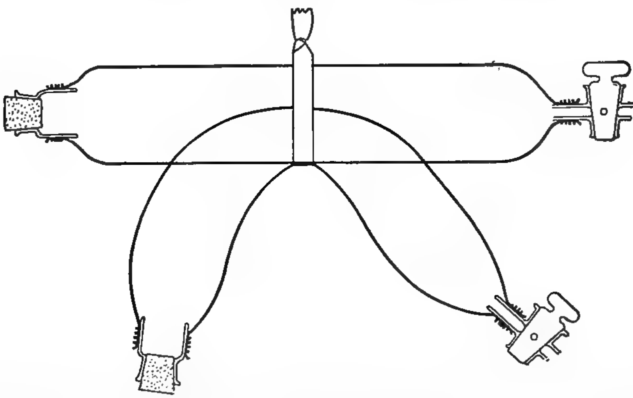


FIG. 41.—ARRANGEMENT FOR DEMONSTRATION OF OSMOTIC TURGESCENCE; $\times\frac{2}{5}$. Details in text.

in the upper part of the figure. The whole arrangement may then readily be preserved, always ready for use, in a bottle of molasses, as described for the demonstration osmoscope (page 146).

Both PFEFFER'S and DE VRIES' methods of determining the osmotic pressures in plants being indirect or somewhat complicated, even though accurate, one naturally asks whether there is not some simpler and more direct, even though less accurate method. Such a method, albeit but crude, does exist, and may be employed by the student as follows:

SUGGESTED EXPERIMENT. Select a slender, soft-textured, turgescent structure, such as a flower-stalk of Tulip or Hyacinth, a petiole of *Oxalis* or *Tropæolum*, and mark off by waterproof India ink a definite length, preferably 100 mm., leaving in addition some 10 mm. at each end. Plasmolyze this by some hours' immersion in a normal solution of salt, and then measure the distance apart of the marks. Clamp one end firmly between bits of cork, and, by a similar arrangement, attach to the other end a small scale pan. Suspend the piece in front of a suitable measure and add weights to the pan until the marks are brought to their original distance apart. Then calculate the original diameter of the piece, and determine from these data the power in atmospheres which must have been exerted by the osmotic pressure. (Further particulars are given by DETMER, 154, or by DARWIN and ACTON, 130.) The student should also note PFEFFER'S gypsum-cast method described in the latter work, 131.

Studies like the foregoing sooner or later bring up the question whether all protoplasmic membranes are semi-permeable, and whether the same membranes are constantly semi-permeable under all conditions. The subject is rather difficult of experiment, and the student had better inform himself upon it through the literature, though there is one phase which he can readily settle for himself as follows:

SUGGESTED EXPERIMENT. Mount in water for the microscope a bit of living tissue having a colored cell-sap, such as lower epidermis of *Tradescantia discolor* (or *T. zebrina*), or even a thin slice of very red Beet; place the slide on the temperature stage, and, raising the temperature gradually, note the degree at which the colored sap begins to come out, and interpret this phenomenon.

A crude demonstration of the same thing is given thus: take two similar small pieces (say cubes of 1 cm. side) of red Beet, boil one for a minute, and place the two side by side in vessels of pure water; observe the difference between them after some hours.

The student should now recall or look about for familiar manifestations of osmotic phenomena, and then should interpret these in terms of osmotic pressures, not simply in a general way, but upon a molecular-pressure basis. Following is a list of some of the more striking of these, several of them offering excellent material for demonstration or experiment of a simple sort, and the student should try to add others.

FAMILIAR OSMOTIC PHENOMENA. These are: (*a*) the turgescence of soft tissues as already demonstrated; (*b*) the power of soft tissues such as young roots, or some kinds of Fungi, to lift heavy weights, break pavements, force apart stones, etc.; (*c*) the crisping of celery, cucumbers, etc., placed in water; (*d*) the formation of a juicy syrup from fruits on which dry sugar has been placed; (*e*) the bursting or collapsing, respectively, of berries cooked with little or much sugar in preserving; (*f*) the plumpness of raisins or currants when cooked, though collapsed when dry; (*g*) the powerful swelling of soaking seeds (in part from osmosis, but in part from imbibition);

(*h*) the germicidal power of strong solutions of the non-poisonous and nutritive sugar, molasses, etc.

It is entirely possible to measure the force of the osmotic process in some of the above cases. Thus it may be accomplished for *b* by jackets of wood held together by spiral springs or even by rubber bands, the roots being later replaced by weights hung from one jacket. For *g* the seeds may be made to break thick glass bottles, or may be made to record a part of their pressure by an apparatus invented by MacDougal, 176, though a simple dynamometer devised for the purpose by Richards (Torreya, 1, 1901, 8) is erroneous in principle (the same journal, 47, 70); a variety of other experiments upon this subject is given by Osterhout in his "Experiments with Plants"; *h* may be very neatly tested by making up a series of solutions of sugar, *e.g.*, molasses, shaking into each the same quantity of yeast, and noting in which solution fermentation can just take place.

There are some special phases of absorption to which the student should give attention, especially the occasional, and somewhat pathological, absorption of water by green leaves and stems, and the important absorption by special structures in epiphytes. Absorption by cut shoots will be considered later under Transfer.

So much for the general and physical aspects of osmotic absorption. We turn now to consider the actual absorption of water by the common land plants, and naturally the first problem to present itself is this:

In what quantity and under what pressures do roots osmotically absorb water?

It is not experimentally practicable to test the individual absorbing rootlets, but the collective action of these, as manifest by the quantity passed from root to stem, may be determined.

Experiment. Select a vigorous single-stemmed, firm-textured plant, and cut away the stem near its transition to root, preferably about 2 cm. above the surface of the ground; over the stump slip a tight piece of rubber tubing projecting a centimeter beyond; into the projection insert a tight stopper containing a slender bent tube which leads to a measuring-glass containing some oil (to prevent evaporation). Water the plant properly (page 40) and observe, with calculation for that left in the tubes, the amount of water exuded by the plant.

Experiment. To the stump of a similar plant attach by a pressure-tight joint a suitable pressure-gauge, and, properly watering the plant, observe the pressure developed.

Materials. These are fully described, with quantities and with directions as to methods of study, precautions, etc., by MISS ECKERSON in the Botanical Gazette, 45, 1908, 50. In this paper it is shown that the common greenhouse plants differ widely in their root-pressures, and still more widely in their exudation quantities, while some, for one reason or another, are impracticable for this study. For exudation quantity the best plants, in order of excellence, are *Begonia coccinea* (though, it may be added, many species of *Begonia* exude large quantities of water, much larger than any other herbaceous plants known to me), *Senecio Petasitis*, Fuchsia, and Marguerite. For root-pressure the best plants, taking account of high pressures, ease of manipulation, and commonness of occurrence, are Fuchsia, Marguerite, Horseshoe Geranium, though *Salvia involucrata*, Tomato, Heliotrope, String Bean, and *Senecio Petasitis* also give high pressures. In general the highest pressures are attained in spring, and on the morning of the second day of the experiment.

DEMONSTRATION METHODS. For exudation an effective arrangement consists of a glass tube, the diameter of the stump, attached upright by rubber tubing thereto; the rise of the sap is then very plain. For the demonstration of pressure the small gauges are not impressive, and a better arrangement consists of a large open gauge of the same general form (including the bulb), made of small-bore (2–3 mm. diameter) barometer tubing, long enough to show an atmosphere of pressure; this gives a demonstration leaving nothing to be desired, provided that a plant of ample exudation quantity be used.

An arrangement for making root-pressures self-registering, using an open mercury gauge with a float which connects with a recording drum, is described by M. B. THOMAS in the Botanical Gazette, 17, 1892, 212. It does not, however, separate pressure from quantity nor allow for increasing weight of the mercury column. Compare also BARANETZKY'S apparatus mentioned by DETMER, 205. An instrument, called a pinometer, for observing simultaneously root-pressures and stem suction is described by DARBISHIRE in Botanical Gazette, 39, 1905, 356, and an arrangement for replacing root-pressure for severed roots is described by BLODGETT in Journal of Applied Microscopy, 5, 1902, p. 1988.

ROOT EXUDATION AND PRESSURE QUANTITIES. In the paper by MISS ECKERSON, above cited, it is shown that, for herbaceous greenhouse plants, the exudation varies from 1.5 cc. through a mean of 26.83 cc. to 263 cc., whence may be derived a conventional expression of 1–25–300 cc. For the same plants the root-pressure varies from .323 through a mean of .92 to 1 605 atmospheres, which may be expressed conventionally as .3–.9–1.6 atmospheres. The mean pressure of .9 atmospheres equals 13 pounds to the square inch.

PRESSURE-GAUGES. Those available for physiological purposes are of three types. *First* are the metal gauges of the steam (Bourdon) type, in which the straightening of a bent flat tube under internal pressure is made to move a pointer around a circular dial. These have been extensively used in measuring sap-pressures (*e.g.*, VINES, Annals of Botany, 10, 1896, 291, and Bulletin of the Experiment Station of Vermont, No. 103, 1903), but they are only useful where large quantities of liquids are concerned.

Second are manometer tubes, containing mercury in a U-shaped bend which is pushed down one arm and up another by the pressure. These are of two types, open and closed as to the distal end. The former are the more striking and easy of manipulation, but they require a larger quantity of water than is always available, and moreover if the pressures are considerable, must be of a very inconvenient and break-tempting length. Hence the latter or closed type are far superior for such purposes as the present. The best form I have been able to develop, one which gives correct readings with very small quantities of liquid, is among my normal apparatus (page 46) and is shown by the accompanying figure (Fig. 42). It is made of small-bore (.5 mm. or less) barometer tubing, of the form, relative length of arms, and position of reservoir bulb shown in the figure, these details being of consequence to its ready filling. The long arm is graduated from above downwards to admit of ready computation of the air column, and the short arm is provided with three glass sleeves, which serve, when cemented over one another and over the gauge with sealing-wax or shellac, to make the gauge large enough for ready attachment to larger stems. The gauge, after being cleaned and dried by aid of alcohol, is filled thus: the short arm is placed in boiled (air-free) water, and clean dry mercury is admitted through the longer arm until the gauge is filled from end to end, when air is admitted above and the mercury allowed to find its natural level. Then a piece of closed rubber tubing (that through which mercury is admitted, or a pipette bulb) is placed on the upper end, and, by a quick compression, the air column is forced down around the bend to the bulb and allowed to spring as quickly back, when a part of the mercury will be forced out and replaced by water, which should fill somewhat less than half of the bulb. Next the air in the long arm is to be dried (for presence of vapor-tension causes an error difficult to estimate); a closed rubber tube or pipette bulb is filled with water and placed over the lower end, where it is gently pressed so as to force the air out through a rubber tube into a chamber containing calcium chloride and phosphorus pentoxide, into and out of which it is moved a few times when it becomes thoroughly dry. The connection is then severed, the exact height of the vertical mercury column, the room temperature, and (for exact work) the barometric pressure are carefully noted, and the

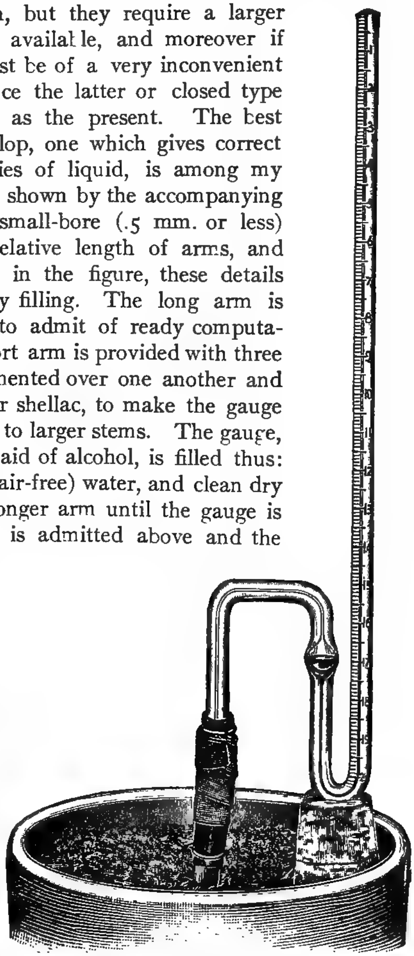


FIG. 42.—HIGH-PRESSURE MANOMETER; $\times \frac{1}{2}$.

upper end is at once sealed by a drop of heated shellac applied from a knife point. The gauge, still standing in the water, should now be put aside for some hours to allow the shellac to harden thoroughly. The short arm is then attached to the plant or other object by a pressure-tight joint (described in Part III) in the usual way. The pressure developed is of course calculated exactly by BOYLE'S Law. In exact work correction is not to be made for the capillary depression of the mercury, but must be made for the weight of the mercury column at the close. If at the close of the experiment the pressure is released gradually, the gauges may be used repeatedly without refilling; the shellac may be removed by alcohol. The drying of the air is a refinement which may be omitted in elementary or other ordinary work, since the error from moisture, especially if the gauge is filled in a dry room, is ordinarily negligible. *Third*, though useful only for some demonstration and elementary uses, there is the simplest possible form, a straight closed tube in which the rising liquid compresses the air, the pressure being calculated by BOYLE'S Law. The tube should approximate in external diameter to the stem, to which it is attached by the usual pressure-tight joint, but its bore should be rather small so as not to require much liquid to fill it. It should be about 10 cm. long and drawn out at one end to a conical tip ending in a very slender open point which is sealed (with precaution against heating the air in the tube) when the liquid commences to rise. A millimeter scale should be attached, and in making the calculations the conical end is treated as a cone, whose capacity is one-third that of a cylinder of the same height and base. A more convenient form of this gauge would be one with a glass stop-cock at the upper end, but it is almost impossible to make such stop-cocks tight against prolonged internal pressure. Such gauges are liable to errors from (*a*) vapor-tension, (*b*) disappearance of some of the air by solution in the water under increased pressure, and the escape of some air from the ducts into the tube. The three former may be approximately determined and compensated from the correction tables in Part III, but as the errors balance one another more or less, for demonstration or qualitative work they may be ignored.

ERRONEOUS USES OF PRESSURE-GAUGES. In a number of the current text-books, and in some more special works which should know better, open gauges of considerable bore are recommended, and these commonly require very considerable quantities of water to push the recording column of mercury to any considerable height. Now it frequently happens, as MISS ECKERSON'S table, earlier cited, will show, that the quantity of water exuded is very small, though its pressure is high; in such a case there would only be water enough to push the mercury column a short distance, which would be interpreted, but wholly erroneously, as meaning a low pressure. Correct pressures can be recorded only by the use of gauges requiring quantities of water so small that full pressures can be registered by inappreciable amounts, as is the case with the manometer above described.

The student should now inform himself as to the quantities of known root-pressures and exudation.

In comparing the structures of osmometers with the osmotic apparatus of the roots, there appears one striking structural difference, namely, in the osmometer there is but one chamber in which the solution works directly against the gauge, while in the roots the solution is enclosed within cells and releases pure water under pressure into the ducts. The physical conditions whereby this water is thus released are not understood, but the student should make sure he comprehends the osmotic problem involved, the available evidence as to whether root absorption is purely physical or in part physiological, and the attempts which have been made to solve this problem.

The study of the absorption of soil water by roots brings us into contact with the structure and properties of the soil. This subject is a vast one, of which our knowledge is still comparatively scant, but its scientific interest and economic importance are leading to its very active study at the present time. Indeed, it already has methods and a technique of its own, making it, like bacteriology, in practice a separate department of investigation. Its experimental study is hardly practicable to any extent in this course, but if the student is able thus to follow it, he will be aided by the following suggestions:

Experimental Study of Soil Physics. The principal topics include (*a*) mechanical structure and its analysis, effected either by a sifting-and-floating or by a centrifugal method; (*b*) aeration capacity and air movements; (*c*) water absorbing and holding capacity, and movements of soil water; (*d*) absorptive capacity for gases and special minerals; (*e*) temperature conditions and their relations to air temperatures; and (*f*) adsorption, or the aggregation of materials in solution towards solid bodies. The subject approaches close to the chemistry of soils, on which some comments will be found a little later. The various methods of study of soil physics are given with the greatest fullness in various Bulletins of the Division of Soils of the United States Department of Agriculture, where the subject has been, and is being, studied with the greatest energy and success, and some of the State Experiment Stations have also contributed much to the subject. A full account of these matters is given also in the very excellent work of Hilgard, "Soils" (New York, Macmillan Co., 1906), more briefly in King's "The Soil" (New York, Macmillan Co., 1899), and in Warington's "Lectures on Some of the Physical Properties of Soil" (Oxford, Clarendon Press, 1900). There are some simple methods of demonstrating the most important of the above-named properties in the Year-book of the United

States Department of Agriculture for 1905, 267, in OSTERHOUT'S "Experiments with Plants," and in DETMER, 244. There is a full treatment of the important matter of Adsorption by TRUE and OGLEVEE in the Botanical Gazette, **39**, 1905, 1. A special recording thermometer for soil studies has been invented by MACDOUGAL (Journal of the New York Botanical Garden, **3**, 1902, 125).

The foregoing studies should make clear to the student the physical nature of water absorption, and we approach now the problem of the absorption of minerals. First of all we must make sure of the facts before we attempt their interpretation, and the problem is presented:

What quantities and kinds of mineral matters occur in common plants?

EXPERIMENT. Select some typical herbaceous plant and, cutting it off close to the ground, quickly weigh it to a decigram. Place it in a suitable dish in the drying-oven until it ceases to lose weight (requiring two or three days), then weigh again, which will give the percentage of water originally present. Then place the dry material in a weighed platinum crucible, fused quartz evaporating dish, or even a piece of hard-glass tubing, and, under a proper hood, gently burn away the organic matter, and determine the weight of the remaining ash.

MINERAL QUANTITIES. A comparison of many tables dealing with the composition of plants has shown that ordinary herbaceous plants approximate conventionally to 90% water, 8% organic matter, and 2% mineral matter.

The determination of the kinds of the minerals left in the ash involves chemical analysis of the soil, an operation rather impracticable here, though some qualitative determinations can readily be made by methods given by DETMER, 79. But the student should inform himself upon the results of some of the many analyses which have been made, and which are summarized in JOST'S and PFEFFER'S works. In this connection he will find that certain minerals occur constantly, and hence appear to be indispensable, in the higher plants, while others are incidental. This involves the inquiry as to the use or meaning to the plant of the indispensable minerals, a subject which is best investigated through water-culture. The methods of water-culture have already been described, and the student may profitably apply them to the present subject after the following:

SUGGESTED EXPERIMENT. Using the water-culture methods earlier described (page 116), determine what effect is produced upon the growth of Barley or Oats by a complete nutrient solution in comparison with a series of solutions, each of which lacks respectively one of the indispensable elements, while one lacks them all. *Or* the student may employ more elaborate methods permitting a more extended observation, and he may profitably use for comparison the fine water-culture diagram of ERRERA and LAURENT (page 23, note). After the plants have grown to near their limit, they should be examined for any anatomical differences they exhibit.

The student should now inform himself as to our present knowledge of the selective power of the roots for particular minerals, and of the uses or significance of each of these minerals to the plant. Upon the latter subject he will find a very valuable paper by LOEW on the "Physiological Rôle of Mineral Nutrients in Plants," in a Bulletin of the United States Department of Agriculture, 1903. This will lead naturally to a consideration of the chemistry of soils, which is, however, a subject impracticable of experimental study in this course. But the student should inform himself upon its important phases through the literature, which is comprised in the same works as those mentioned above under Soil Physics; and he should especially note the origin, in the soil, of the minerals used by plants, the mode of renewal of supply, and the comparative amounts dissolved by water from different soils. Another important phase of the subject concerns the possible secretion of acids from the roots for dissolving mineral matters otherwise insoluble; this involves the study of corrosion phenomena, upon the experimental study of which there are directions in DETMER, 241. Allied to this is the matter of the oxidizing power of roots through enzymes, and its significance, which is fully discussed in the Journal of Biological Chemistry, 3, 1907, by SCHREINER and REED, by whom, also, valuable and striking experimental methods of demonstrating this power, through use of phenolphthalin and other indicators in water-culture solutions, have been worked out and will later be published. The possible excretion of other organic substances by roots will be noted later under Excretion.

The student should here also ascertain the present state of knowledge or opinion as to the relative values of physical and of chemical properties of the soil in determining plant habit and distribution. He will find a strong argument against the prevailing views given by FERNALD in Rhodora, 9, 1907, 149.

Another phase of absorption is that by parasites, saprophytes, and insectivorous plants in taking the organic matters from their respective sources, and upon the physics of this absorption the student should also inform himself.

Such are the modes of absorption of water and minerals. It remains to consider the absorption of gases, a matter of vast importance because of the indispensable part played by carbon dioxide and oxygen in the economy of the plant. Nitrogen, of course, is not here in question, since it is absorbed only in combined form in solution. A certain restricted amount of all three gases is absorbed in solution in water through the roots, but this is insignificant in amount, and, moreover, is stopped in the vessels, thus explaining the gases always present there. For its principal supply of carbon dioxide and oxygen, therefore, the plant must draw upon the great reservoir, the atmosphere, which it must absorb through its aerial parts. For a full understanding of this subject the student must now renew his acquaintance with *the structure of the gas-absorbing parts, including the aeration system, of the higher plants, together with the stomata and lenticels, their distribution and connection with the intercellular system, the morphology of the latter, its extent, its continuity throughout the plant, its presence in compact tissues, and the character and condition of the cells in contact with it.* All these matters the student should understand with a definiteness permitting the construction of a generalized or conventional diagram of the aeration system of the plant from root tip to stem tip and leaf. So important to our present subject is the continuity of the aeration system and its connection with the stomata, that it deserves some experimental study, which may take the form of demonstration as follows:

SUGGESTED EXPERIMENTS. Detach from its plant a long-petioled loosetextured leaf, and over the cut end slip a tightly fitting rubber tube; through this, by aid of a blast or air-pump (*e.g.*, a bicycle pump), force air into the leaf; then plunge the latter under clear water in a glass vessel and observe closely the surface of the leaf. Also by a similar method force air through a long internode of some soft-textured stem, a considerable length of root, or other selected part, holding the free end under water.

The best material for this experiment is afforded by the long-petioled floating leaves of water-plants, some of which are so porous that air can almost be blown through them by the mouth. *Limnocharis Humboldti*, commonly grown in greenhouses, is good. Next to water-plants come some of the Begonias, then Rubber Plant and Orange, while the tropical house plants *Jacobinia magnifica*, *Codiæum aucubæfolium*, and *Clerodendron Thompsonæ* are also good.

Since this experiment is liable to be marred by closing of the stomata, or by clogging of them through water entering by capillarity, it is best to ensure their open condition by first keeping the plant for a short time in a bright light, well watered, and in a somewhat humid atmosphere; while it may also help to keep the stomata open if the leaf is cut off under water, thus preventing entrance of air into the ducts. Also the clogging of the openings may be prevented by having the air-current in action before the leaf is plunged under water. It is these difficulties which have led to the recommendation of a method, *e.g.*, Detmer, 172, whereby the air is drawn through the leaf and escapes under water from the petiole; and other modifications have been described.

Turning from the gas-absorbing system to the gases absorbed, the student should now work out, and should tabulate for carbon dioxide and oxygen (and preferably also for nitrogen), their chemical composition and molecular weights, their properties and affinities, their relative diffusibilities and solubilities, and their proportions in the atmosphere. He should then turn particularly to their remarkable power of diffusibility, so important in this connection, and his understanding of this matter will be much aided by some practical study of the phenomena, which he may well bring before him as a problem thus:

What are the principal phenomena, and the physical explanation, of the diffusion of gases?

Experiment. Prepare four tubes of rather thick glass, of some 10–12 mm. bore and 8 cm. length, with one end ground flat across. Cut two discs of parchment paper, one of thin rubber sheeting, and one of a leaf having stomata on only one surface, such as *Hedera Helix* (English Ivy), *Codiæum*, or *Ficus elastica* (Rubber Plant); seal these air-tight by sealing-wax to the ground ends of the tubes (Fig.

43), the leaf with its stoma side outward; fill them all with carbon dioxide by dry displacement, and support them upright in a dish of mercury. Keep one of the paper discs moist by wet filter-paper on one side; observe and interpret the results. After a pronounced effect appears, requiring one or two days, place a piece of caustic potash under each tube. Later the same tubes may be filled with hydrogen and then with oxygen.

Or an instructive result is given by simply binding a loose-fitting piece of thin rubber sheeting tightly to the mouth of a test-tube containing carbon dioxide. The parchment paper may be tied on while wet, if dried before use.

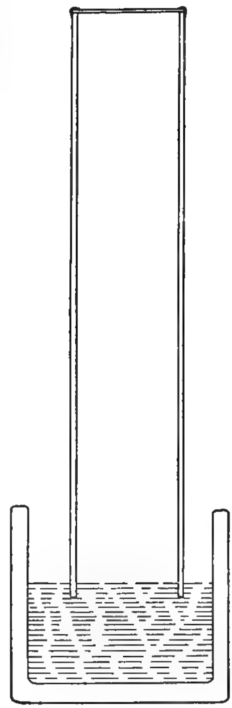


FIG. 43.—DIFFUSION TUBES; $\times \frac{1}{1}$.
The heavy black represents sealing-wax; further explanation in text.

The student should now make sure that he understands *the physical nature of the diffusion of gases, the kinetic theory of gas-pressure, the source of the energy causing it, its molecular basis, the relative pressures exerted by equimolecular volumes, the effect of temperature upon volume and pressure, and the diffusion movements of one gas in a mixture in relation to the others of that mixture when the gas is being either absorbed or released at some particular place*, for such conditions are actually found in the intercellular passages of the plant.

Returning, with our new knowledge, to the structure of the plant, a first and natural inquiry to arise would be this:

Does carbon dioxide actually enter the plant through the stomata?

This may be determined, indirectly but conclusively, by observing whether any photosynthesis can take place when the stomata are blocked.

EXPERIMENT. Select a plant of good photosynthetic power, but having stomata only upon one surface, *e.g.*, *Fuchsia*, *Abutilon*, *Euphorbia*, *Senecio*, and keep it in darkness over a day. Then cover one-half of the stomatal surface with a thin layer of vaseline, or, better, with a mixture of three parts cocoa-butter with one part beeswax, applied somewhat warm, which has the advantage of caking off readily in cold water; also as a control cover half of the astomatal surface of another leaf with the same mixture. Leave them for two or three hours in light, then remove the vaseline or wax, blanch them, and apply the iodine test. *Or* the leaves may be left with the coatings for two days in light without the previous period in darkness.

This method was described by Stahl in the Botanische Zeitung, **52**, 1894, 129, where fuller particulars are given, and independently by Blackman in Science Progress, 4, 1895, 30.

The ready entrance of gases through stomata in the face of the very small size and relatively small number of the latter (compare the Stoma Quantities later) suggests an inquiry into the facts about the passage of the gases through small apertures, a matter of importance involving rather unexpected phenomena, which the student should study through the works on the subject, above all in the paper of Brown and Escombe in the Annals of Botany, **14**, 1900, 537.

The mode and impelling energy of the entrance of gases into the intercellular system of the plant being understood, it remains to consider the absorption from the air-passages by the living cells, which present moist membranes to the gases. That carbon dioxide can pass readily through moist membranes has already been shown by an earlier experiment (page 164), and may readily be demonstrated by binding a moist membrane over a vial of lime-water which is then inserted into a vessel containing a considerable percentage of carbon dioxide. Various considerations show that the carbon dioxide here goes into solution, and then it passes by diffusion to the interior of the cell.

Literature of Absorption. There are, of course, the usual general works of Pfeffer and of Jost, but the special papers have been cited under their respective sections.

7. TRANSPORT.

Having considered the absorption of substances into the plant, we must next study the mode of movement, or *transport*, of those substances from place to place. In practice it is convenient to separate the subject into two parts,—the movement of pure water through the plant, a process sometimes called *transfer*, and the movement of elaborated food substances from place to place, commonly called *translocation*.

(*a*) *Transfer.*

Turning first to transfer, it is plain that a certain amount thereof is provided by osmotic movement from cell to cell, but this is wholly inadequate to explain the rapidity with which great quantities of water are moved throughout the plant to make up the loss from transpiration, a process soon to be studied. Unfortunately our knowledge of this subject, despite much study, is still very imperfect, and the experimental study is mostly in a corresponding condition. The student can doubtless spend his experimenting time to better advantage upon other subjects, working this out through the literature, but if he can follow it practically he will be aided by the following:

SUGGESTED EXPERIMENTS. *Rate of Ascent.* Cut under water a translucent colorless shoot, such as *Impatiens;* transfer it to a strong solution of eosin or methyl green (or red ink), and observe the rise of the color in the fibrovascular bundles to the leaf. *Or* use shoots having pure-white flowers, and note the time needed for these to show the color. *Or*, and better, follow SACHS' method of watering the plant with lithium nitrate, which can readily be recognized by the spectroscope in samples of tissue taken at different heights. (Note fuller directions in DETMER, 233; and there is very valuable matter in a paper on "Color in Plants" by KRAEMER in Bulletin of the Torrey Botanical Club, 33, 1906, 77.)

Path of Ascent. Select a shrubby plant, such as a rose-bush; remove the bark on one branch in a ring all around, 1 cm. long, and, to prevent drying out, cover the place with vaseline or grafting-wax. Also, remove a similar ring on another branch, but including also the wood for 2 mm. deep, and cover as before. Note the effect upon water transfer as shown by the wilting of the leaves.

After the shoot placed in eosin, as described above, shows signs of red in the leaf-veins, cut cross-sections at various heights and observe the place of occurrence of the color. Also, in the experiment with white flowers observe the exact location of the red color.

Ascent in Lumina or Walls. Fill the lumina without injury to the walls in a shoot as follows. In a water-bath in a shallow dish (such as a paint mixer 5 cm. in diameter) warm cocoa-butter colored by lampblack until it just melts (which will occur at 33°, a temperature harmless to the plant), and warm pure water in a similar dish (for a control). Select a convenient herbaceous plant, which has been allowed to become somewhat dry (to develop negative pressure explained below), bend its stem at one point beneath the melted cocoa-butter and sever it there, cutting another similar shoot under the water. At once transfer both to a dish of fresh water, and, as soon as the butter is hardened, scrape a thin layer from the cut end in order to get rid of surplus butter and to expose the walls as well as the filled lumina to the water. Note the comparative rate of wilting of the two shoots. Or this can be done also with gelatin as described by DETMER, 227.

Motility in Wood. Cut a fresh cylinder some 5 cm. long and 1 cm. diameter from growing pine or other coniferous wood, and soak it in pure water. Then, shaking it clear of all water, place a drop on the top and note any result at the bottom; also invert the piece and repeat the test. Over one end of a similar piece 1 cm. long fit a stout rubber tube through which clean water is to be forced under a known pressure, obtained either by a water column, or preferably by mercury pressure in a U tube; observe the amount which filters through the wood in a given time. Then in precisely the same way test a cylinder cut in the tangential, and one cut in the radial, direction.

Continuity of Vessels. Stir some fine cinnabar in distilled water, and filter repeatedly until a mixture is left in which the cinnabar will not settle for days. Then, using the apparatus of the preceding experiment, force the mixture under pressure into longitudinal cylinders of different kinds of wood, and note whether the passing water contains the cinnabar. Later make longitudinal sections of the wood, and note under the microscope the distribution of the cinnabar.

Negative Pressure in the Vessels. Select two similar herbaceous plants, and keep one dry (unwatered in a dry room) and the other very moist (by ample watering in a damp chamber or bell jar in darkness). Cut a shoot of each (*a*) under a strong solution of eosin, (*b*) under mercury, (*c*) under melted colored cocoa-butter, and at once make longitudinal sections and examine microscopically with reference to penetration of the substances into the ducts. If a translucent stem is used, the height to which the liquid jumps can be viewed directly by holding up to the light. Also, select a half dozen different herbaceous plants, allow them to become partially dry, then cut from each two shoots, one under water and one in air; place both in water and observe the comparative rate of wilting. The cutting in all these cases is best effected by use of small pruning shears.

This negative pressure in the conducting system of plants, due to rarefaction of the air-bubbles in the vessels under influence of very rapid removal of water from the stem by transpiration, is a matter of much practical importance in experimentation, because, when the stem is cut in air, this rushes into the ducts and interrupts perfect conduction after the shoot is placed in water. This difficulty is the greater the more rapidly water is being removed from the plant, and disappears with greatly reduced transpiration; but in practice it is safest for the student to cut under water all shoots or leaves intended to be kept fresh in water.

Tension of Water. Over the large opening of a thistle-tube tie tightly a piece of soaked parchment paper; fill the tube with boiled and cooled water, and support it upright with the small open end standing in mercury; stand the whole arrangement in a bell jar which can be exhausted to a vacuum, and observe any changes in the levels of the liquids. Also replace the paper by a tightly inserted, stiff-leaved shoot, and observe as before.

As commonly tried in the laboratory, without removal of atmospheric pressure, this experiment is quite valueless for a test or demonstration of the tension or cohesion power of water and of mercury, for the rise of the mercury is due to nothing but the external atmospheric pressure as the water is removed by evaporation. To demonstrate any proper tension, and hence any real lifting power of evaporation or transpiration, it is necessary either to use a tube which will show more than one atmosphere of mercurial pressure, or, better, to make the test in a vacuum. Compare VINES in Annals of Botany, **10**, 1896, 291, and the practical direction for experimental study of this subject given by STEINBRINCK in Flora, **94**, 1905, 466, and a mode of making it self-registering by SCHOUTEN in Flora, **97**, 1907, 118.

Participation of Living Cells. Select a shrubby plant, such as a rose, and from one branch held vertically remove a ring of bark for 1 cm.; just below this build on the stem by modelling-clay a close-fitting and deep funnel, 1 cm. deep, and into this pour hot water, renewed several times. On another branch place in the funnel a concentrated solution of picric acid (very poisonous to plants as well as to men), once or twice renewed. Note in both cases the effect upon transfer, as shown by wilting of the leaves. These two methods kill the living protoplasm, but in different ways.

The student should now work out the present state of our knowledge of this subject from the literature, giving special attention to *the structure of wood, the mechanical problem involved in the ascent of sap, and the principal theories developed to explain water transfer, with the energetics of each.* As there are many modifications of view of the subject, he may well confine himself to the four principal theories: (*a*) the older capillary; (*b*) SACHS' imbibition; (*c*) the propulsion theory of GOD-

LEWSKI; and (*d*) the traction theory of DIXON and JOLY. All of these he should expound clearly in a suitable essay.

LITERATURE OF TRANSFER. The literature of this perplexing subject was admirably summarized, and the knowledge of the subject discussed, down to 1888 by H. MARSHALL WARD in "Timber and Some of its Diseases" (London, Macmillan, 1889). Since then the most important works have been STRASBURGER'S "Die Leitungsbahnen der Pflanzen" (of which there is a good review in Annals of Botany, **6**, 1892, 217) and the very important works by DIXON and JOLY in the Annals of Botany, **8**, 1894, 468, in the Proceedings of the Royal Society, **57**, 1894, 3, and in the Philosophical Transactions, **186**, B, 1895, 563. There is a summary of an important discussion upon the subject in the Annals of Botany, **10**, 1896, 630. DIXON has recently answered objections and reaffirmed his views in the Proceedings of the Royal Society, **79**, 1907, 41. There is a very complete summary of the literature down to 1902 by COPELAND in the Botanical Gazette, **34**, 1902, 161, a synoptical summary by WIEGAND in the American Naturalist, **40**, 1906, 409, and a brief summary of recent literature by BARNES in Botanical Gazette, **42**, 1906, 150.

(*b*) *Translocation.*

Our knowledge of the movements of plastic or food substances through the plant, especially as concerns the energetics of the process, is very incomplete, and the experimental study of the subject is for the most part not profitable for the student of this course. Still he may follow it to some extent through the following suggestions:

SUGGESTED EXPERIMENTS. *Translocation by Streaming.* This limited form of transport may be observed in large streaming cells, in Myxomycetes, and in growing pollen-grains. Compare DETMER, 346. Correlated with this are possible movements in the latex system and those due to growth.

Path of Translocation. This, for the general route, is determined by ringing the bark, *i.e.*, removing a ring all around the trunk, or else by constricting it, which results in an abnormal growth, due to accumulation, above the constriction. Compare DETMER, 351. Here belongs also the difficult subject of the relative parts taken by the sieve system, the starch sheaths, and the cortex, in translocation.

A phase of this subject of special prominence and interest, *viz.*, the translocation of the photosynthate from green leaves into the stem, has already been noted under photosynthesis, where its consideration has some practical experimental value. It may further be tested thus:

SUGGESTED EXPERIMENTS. *Demonstration.* The disappearance of starch from unlighted leaves, or their parts, may be shown very simply by attaching a normal screen to a leaf full of starch, say at 10 or 11 A.M., leaving it for some hours, and later applying the iodine test. This, however, by itself proves only the disappearance of starch as such, and not necessarily the removal of its substance. Here it is important to note the reason for the appearance of the starch at all in the leaf, *viz.*, its use as a reserve form when the sugar of the leaf is accumulating to an osmotically unfavorable amount.

Amount Translocated. This is very readily settled by use of the leaf-area cutter and method, used in a manner reciprocal to that described for photosynthesis.

Relation to Temperature. This is determined, though at expense of much effort, by use of the leaf-area method applied to plants kept for some hours at very different temperatures. Several similar plants are taken when the leaves are full of photosynthate, say at noon; a number of areas are cut from each one; the plants are then placed for some three or four hours in darkness, each in a different temperature; then areas equivalent in number and position to those first taken are removed from them, thus supplying data for a determination of this question.

A point of practical experimental value in this connection is this: that at high temperatures, say above 25° for most plants, the translocation is so rapid that no starch at all forms in the leaf, for which reason all experiments involving starch formation should be carried on at temperatures not above 25°, and much better at 20°–22°.

The student should now inform himself through the literature upon *the facts of translocation, including its importance, its paths, and its energetics*, the latter involving diffusion, probable special pressures, and possible peristaltic actions.

Closely connected with Translocation is Accumulation of reserve substances, and Secretion of materials of special function already noted in another connection. The student should here work out the method of the transformation of substances from the soluble to the insoluble form, the effect thereof upon the continuity of the diffusion streams, and the physical method by which the substances are removed out of the protoplasm.

LITERATURE OF TRANSLOCATION. The general works of PFEFFER and of JOST cover this subject well. It comes also very closely into contact with Conversion, earlier studied, and some of the literature there cited (page 121) applies here also.

8. ELIMINATION.

Having studied the modes by which substances are absorbed into and transported through the plant, we have next to consider the manner of their elimination from the plant. Of this there are three phases, *viz.*, (*a*) the elimination of water as vapor, or *Transpiration*, the most striking of all; (*b*) the elimination of liquid water, or *Guttation;* (*c*) the elimination of waste matters, or *Excretion.*

(*a*) *Transpiration.*

It is a sufficiently familiar fact that ordinary plants give off large quantities of water-vapor through their leaves, though if any further evidence thereof is needed, it can be supplied by simple experiments described in a note (under Demonstrations) below. We may therefore proceed directly to the obvious first problem of our study, which must be this:

In what quantities is water transpired from ordinary plants under usual conditions?

This may best be determined by the delicate quantitative test of weighing, the utilization of which requires that the transpiration shall not be complicated with evaporation from the soil.

EXPERIMENT. Select a convenient single-stemmed potted plant, preferably from the materials mentioned below, and cover pot and soil with vapor-proof coverings, so that all loss of water must be through the leaves and stem. Weigh the plant on a good balance at intervals as frequent as practicable, watering the plant and renewing the air inside the coverings once a day. Tabulate the loss through two or three days.

PRECAUTIONS. While not essential, it is desirable for the better health of the roots, and hence of the whole plant, to use a method for enwrapping the pot such as will permit a daily renewal of the air. In watering it is a good plan to start by giving the plant only about half its usual amount (since all evaporation will be prevented), and thereafter to add each day approximately the amount it has lost in the preceding twenty-four hours. The weighings should be so arranged as to include the eight hours of greatest daylight (thus being made at 8 A.M. and 4 P.M. or thereabouts), which permits a comparison with the loss by night when the sixteen hours thereof is divided by two.

MATERIALS. These have been studied thoroughly from the experimental point of view by MISS GRACE CLAPP, with results contained in the

Botanical Gazette, 45, 1908, 254. She shows that the most vigorous transpirers, in order of excellence, are Sunflower, Tomato, Lady Washington Geranium, Marguerite, and White Lupine. Some of these, however, have to be raised on purpose or have other practical drawbacks, so that for educational purposes the most available may be considered to be, in order of general excellence, Marguerite, Garden Nasturtium, Lady Washington Geranium, Fuchsia, and *Senecio Petasitis*. This paper also gives full numerical data upon these and the other common greenhouse plants.

ENWRAPPING OF POT AND SOIL. Many and diverse methods of accomplishing this have been described by different students as follows: (*a*) The pot is placed in a glass jar roofed by bored-and-split sheet lead or glass plates, all joints being made tight by wax or cement, or by rubber sheeting. (*b*) The porous pot is replaced by a glazed vessel or by one of metal, roofed over as before. (*c*) The pot is painted completely by melted paraffin, or by a thin coat of modelling-wax, and roofed over by the same materials. (*d*) The pot and soil are enwrapped completely by thin rubber sheeting gathered around the stem of the plant. In all these arrangements the roots are usually watered through a thistle-tube permanently inserted for the purpose, and kept corked. More recently a new method, having high value for some special purposes, has been introduced by WHITNEY and CAMERON, as described in Bulletin No. 23, 1904, of the Bureau of Soils of the United States Department of Agriculture, and by LIVINGSTON in the Plant World, 9, 1906, 62; it consists in the use of wire baskets covered by paraffin, which have also the advantage, for many purposes, of ensuring an even distribution of roots in the soil. A method of covering the soil, by use of cement, where the plants are in the ground and to be studied under a bell jar, is described by CANNON in Bulletin of the Torrey Botanical Club, 32, 1905, 515. A method of avoiding enwrapping the pot at all was introduced by MASURE (BURGERSTEIN, 5), and is employed in the RICHARD registering evaporimeter later described; a pot containing the plant is balanced with another exactly like it, but containing no plant, the evaporation from the two being assumed to be equal; but obviously the method may lead to much error. An ideal method should permit of rapid and neat enclosure of the pot, and should allow of ready daily renewal of the vitiated air of the soil; and these conditions are very well met in the use of the aluminum shells roofed with rubber, which I have described in the Botanical Gazette, 41, 1906, 212; they are now obtainable among my normal apparatus (page 46) and are figured herewith (Fig. 44). Flower-pots are now made so nearly in standard sizes that it is possible to make the shells to fit them closely, and shells are now made for the 3-inch, $3\frac{1}{2}$-inch, 4-inch, and 5-inch sizes. To hold the rubber roof closely to the shell, a narrow band or strap of aluminum, resting in a groove just below the strengthened top of the shell, may be drawn to any desired tightness by a convenient screw-nut, shown on the right in the figure. The rubber roof may be attached to the plant in any of the ways ordinarily used, but I find that, upon the whole, the best method is the following: In the middle of a suitable-sized piece of medium-thick rubber sheeting, a hole a little smaller than the stem of the plant is made with a cork-borer, and a cut is made with scissors from this to the margin of the piece. This rubber is then placed around the stem, the cut edges near the central hole are stretched to overlap a little, they are sealed together with liquid rubber cement (that used in mending bicycle tires, and everywhere obtainable), and are held clasped until this sets. Then a line of the cement is run to the margin, sealing one edge over the other. When fully set the margin of the rubber is clamped to the shell, all surplus

FIG. 44.—ALUMINUM SHELLS, WITH RUBBER ROOF, FOR PREVENTING EVAPORATION FROM POT AND SOIL.

material is cut away, and there remains a very neat and perfectly tight roof, readily removable at any time.

TRANSPIRATION BALANCES. Obviously these should be of a degree of accuracy according with the work to be done. For ordinary demonstration purposes, a balance sensitive to a gram, such as the Harvard Trip-scale (costing about $5.00) or the Springer torsion balance (costing about $14.00), is excellent. Where a more accurate form, necessarily with knife-edge bearings, is used, care must be taken to secure very long pan-hangers to allow room for the plant under the beam. The Gerhart balance, No. 3423 (costing about 115 marks), serves fairly well, but better are the special arrangements described by HANSEN in Flora, **84**, 1897, 355, and by HOEHNEL, figured in BURGERSTEIN'S "Die Transpiration," 8. Still better is a form without an overhanging beam, permitting the use of a plant of any height, and such is figured by PFEFFER, I, 241. An objection to all these balances, especially when many weighings must be made, is the slowness with which the approximate weight is found by use of weights. Spring balances are ideal in this particular, but are far too inaccurate for any but the crudest demonstration use; but sliding weights afford a fair substitute. Following the general form of PFEFFER'S balance, but using a sliding weight for the coarse adjustment, I have designed a new transpiration balance which is among my normal apparatus (page 46) and is figured in the catalogue descriptive thereof.

DEMONSTRATION METHODS. With large classes I have found it most effective to use a large leafy potted plant (see Materials earlier), having pot and soil enwrapped with aluminum shell and rubber roof; it is weighed on a torsion balance sensitive to a gram. After being prepared before the class, the plant is then watered at regular intervals until the class meets again, or for an entire week. If the quantities found to be lost are then exhibited in measuring-glasses, it adds much to the effectiveness. If, however, only the fact, and not the amount, of transpiration is to be shown, then this is very easily effected by simply covering the plant with a bell jar, upon which in a few minutes water abundantly collects. Of course evaporation from pot and soil must be prevented either by enwrapping them or by keeping them outside the bell jar, the stem being passed through a split glass plate; this is effected very conveniently and perfectly by the supported bell jar later described (page 187), for which a substitute may be adapted from any waterproof stiff material (*e.g.*, an indurated fiber saucer) arranged to rest upon the pot. For exhibition to an audience it is possible to place in the bell jar a large disc of paper infiltrated with cobalt chloride, of which the change of color, with accumulating moisture (described later on page 190), can be seen from a distance. The simplest arrangement of all, one very effective for elementary work, is a shoot or a single leaf thrust through a small hole in cardboard resting upon a nearly filled tumbler of water, and covered by another inverted tumbler.

OTHER METHODS OF MEASURING TRANSPIRATION. Weighing is by far the most accurate and satisfactory method of measuring transpiration yet known, but it has one obvious drawback, *viz.*, it is applicable in general only to potted plants. Other methods which overcome this difficulty are the following. *First*, the vapor released by the plant in a closed space is absorbed by a chemical whose increase of weight gives the transpiration; the method is described by DETMER, 214. But it has an inseparable error, —the vapor conditions are not normal in the chamber. *Second*, the relative humidity of a plant chamber of known capacity, in which is a transpiring plant, is determined by a suitable instrument, whence the absolute humidity and transpiration can be calculated. This is recommended, with use of a polymeter, by CANNON, for plants in place in the ground, in Bulletin of the Torrey Botanical Club, 32, 1905, 515. Related in general principle is the method of drawing a known amount of air through a plant-containing chamber over metal tubes arranged to show the dew-point, as described by LEAVITT in the American Journal of Science, 5, 1898, 440. Both methods are somewhat elaborate, and moreover do not allow the plant wholly natural transpiration conditions. *Third*, the water absorbed by the plant is measured or weighed, and is assumed to equal the transpiration, as in the long run it must do. This is accomplished for potted plants by the KRUTITZKY apparatus (figured by BURGERSTEIN, 22); the plant in a glass jar is watered from below by a siphon fed from a movable float, which may be made self-registering. The arrangement necessarily keeps the roots saturated and thus gives conditions inimical to the health of most plants. Far better in every way would be LIVINGSTON'S self-watering arrangement, described in the Plant World, 11, 1908, 39, which could readily be made self-registering (by making the feed-vessel a slender cylinder carrying a float moving a pointer against a revolving cylinder), and which may yet play a large part in the study of this subject. Another arrangement of KRUTITZKY'S, taking a cut shoot and self-registering, is figured by GOODALE, 273. An older arrangement, taking a cut shoot, is VESQUE'S (figured by BURGERSTEIN, 21); the plant and its vessel are balanced with another communicating vessel, and the amount of water needed to keep them in equilibrium is measured as added. The absorption method, however, is most commonly applied to cut shoots or water-culture plants; the cut end or roots of these are sealed air-tight into the neck of a tall vessel having a communicating slender graduated cylinder, in which the loss of water may be read off at intervals. It is illustrated by PFEFFER, 1, 232 (BURGERSTEIN, 18). A method of making this self-registering, by use of a float in the cylinder carrying a pointer against a revolving drum, is described by STONE in Torreya, 4, 1904, 19. The objection to this method consists in the unnatural conditions of the absorption, which makes this transpiration an unsafe index of transpiration under natural conditions, while errors may also arise from the volume changes in the water under varying temperature and accumulation of gas, etc. When the apparatus is made small and the cylinder becomes reduced in bore to a degree sufficient to render the loss of water observable by the eye, these instruments merge over to potometers, which will be described in a later section.

A method of weighing by small hanging springs, read by a horizontal microscope, is described by DARWIN and ACTON, 101, but has marked limitations. This arrangement is made self-registering by LINSBAUER, 39.

In studying the results yielded by the foregoing experiment, and especially in comparing the results obtained by different students, either for different plants or even for different sizes of the same plants, it becomes at once evident that the result must vary with the size and leaf spread of the plant. These differences must be compensated before any comparison of the transpiring powers of different plants can be made, and before any definite statement can be made as to the transpiring power of any given plant. This compensation is best made by determining the leaf area of the plant and reducing the transpiration to grams per square meter per hour, which yields a valuable constant, and this calculation the student should make for all plants that he studies.

Measurement of Leaf Areas. This may most rapidly be accomplished by following the outlines of the leaves themselves, or else tracings of them, by a planimeter, an engineer's instrument which reads off the areas directly. *Or* the leaves may be traced upon cross-section paper and the enclosed areas counted. Better, however, especially if many are concerned, is the plan of tracing the outlines upon one sheet of paper of uniform thickness, cutting the tracings out, and weighing them in comparison with the weight of a definite measured area of the same paper. Instead of tracing the outlines they may, for greater accuracy, be printed under a sheet of glass upon sensitized paper, these prints being later cut out and weighed. There is, of course, some transpiration from stems and petioles, but since this is, relatively to that from the leaves, very small, and, further, since the areas of those parts are in general proportional to those of the leaves, no great error absolutely, and none at all relatively, is made by omitting them from the calculation, though for very exact work they would be included. It is much better to take the area of the leaf as a whole for the standard of comparison, and not the area of its two surfaces, for thus it is possible to keep to the gram-meter-hour system already used for photosynthesis and respiration.

The records of transpiration in the foregoing experiment show notable fluctuations from day to day and from hour to hour, and even a cursory observation thereof will show that the fluctuation is in some measure connected with the immediately surrounding conditions. Hence we are now faced by this problem:

In what measure is transpiration affected by variations in atmospheric conditions, viz., temperature, light, humidity, barometric pressure?

This may be determined most simply by accurate simultaneous observations of the transpiration in comparison with the records of instruments exhibiting the surrounding conditions, a method which is wholly satisfactory only when all the instruments are autographic or self-registering.

Experiment. Place a plant, freshly prepared as for the preceding experiment, under the ordinary fluctuations of the experiment greenhouse. Then, either by very frequent (at least hourly) weighings, with simultaneous readings of the meteorological instruments, or else, and much better, by use of autographic instruments, determine the transpiration of the plant and the contemporary meteorological conditions for a considerable period of time, preferably a week.

Upon certain days alter the conditions in the house to as extreme degrees as practicable towards heat, coldness, humidity, darkness. Then plot the results in a series of graphs arranged one above another, and interpret the parallel and the divergent fluctuations.

TRANSPIROGRAPHS (AUTOGRAPHIC, OR RECORDING, OR REGISTERING TRANSPIROMETERS). Of these several forms have been invented. Some of them, notably those of VESQUE and of KRUTITZKY, register the quantity of water absorbed, and have already been described (page 176). But the more important forms register the actual loss from the potted plant. PFEFFER ("Physiology," 1, 242) speaks of the possibility of using a spring balance, magnifying wheel, and recording drum, but, as I know from trial, no such instrument can be constructed to work with accuracy, since even the best of such balances alter the tension of their springs with use, and also involve irregular friction in the mechanism. A form, taking only a water-culture plant, balanced over a wheel by a submerged float and recording upon a revolving drum, is described by COPELAND (Botanical Gazette, **26**, 1898, 343); another on the same general principle, but taking a potted plant, is described by CORBETT in the Twelfth Report of the West Virginia Experiment Station. An objection to these forms is their comparative unportability and the difficulty of keeping their water at constant temperature, as is essential if the float is not to change its buoyancy. A very different form is the Evaporimètre of RICHARD FRÈRES of Paris, described in their catalogues; it is a balance of adjustable delicacy, recording the movement of its beam directly upon a revolving drum, and using MASURE's method earlier mentioned (page 173), of compensating evaporation from pot and soil. But this instrument has serious limitations in principle and practice. A vast advance upon all earlier instruments was made by ANDERSON in his registering balance, described in Minnesota Botanical Studies, 1894, 177; it is constructed upon a general principle closely followed by my own instrument described below, and has only the demerits that it is costly and must be specially made to order. Another very accurate instrument is that invented by WOODS (Botanical Gazette, **20**, 1895, 473); it is a modification of the MARVIN self-recording rain gauge. Finally I have constructed, and there is offered among my normal apparatus (Botanical Gazette, **39**, 1905, 145), a precision transpirograph, applicable to any good balance, the construction and use of which is as follows. A cylinder, showing prominently in the accompanying figure 45, contains on a spiral track between its outer and an inner wall some 250 spherical gram weights. These weights are steel bicycle balls of one-fourth inch diameter, which weigh almost exactly one gram each, and vary not over one-thousandth of this weight from one another These feed by gravity, one at a time, into a simple releasing valve, so arranged that when acted on by an electromagnet a slide rises and allows one ball to drop through a tube into a scale-pan, a new ball immediately taking its place in the releaser-slide. Attached to the releaser slide is a bar carrying a pen, so adjusted that every time the slide moves, that is, every time a ball is dropped, the pen makes a vertical fine line with chronographic ink upon a record paper attached to a revolving drum. In use the plant to be studied is prepared in the manner usual for transpiration studies, and is balanced

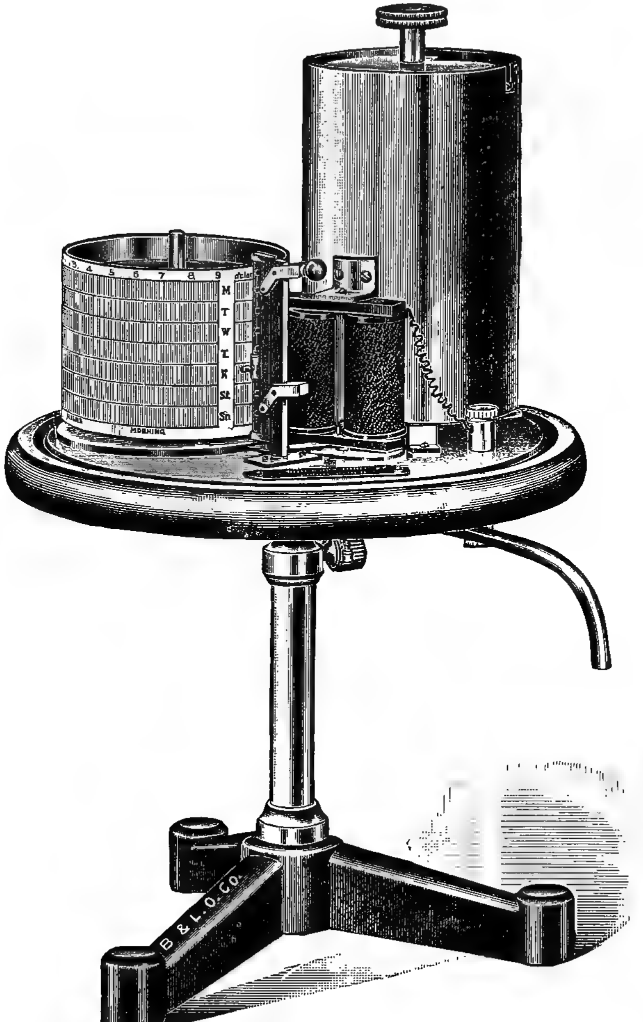


FIG. 45.—PRECISION TRANSPIROGRAPH; $\times\frac{2}{5}$.
Description in text.

on a scale-pan of any good balance sensitive to one gram, while the transpirograph is adjusted beside it. As the plant loses water this pan rises, and at the top of its swing is made to touch a wire so arranged that an electric circuit is thus closed; this excites the electromagnet and thus raises the releaser-slide which drops a ball into the scale-pan, at the same moment making a mark on the record paper. The weight of the ball then depresses the scale-pan, breaking the circuit. This operation is repeated thereafter every time the plant has lost a gram of water. The drum revolves once in twenty-four hours, and the record paper, which shows clearly in the figure, is divided into numbered spaces corresponding to the hours; these spaces are subdivided into twelve parts, each therefore representing five minutes, and these in turn can easily be read by estimation to fifths, or one-minute intervals. It is possible, therefore, to read off from the drum directly the number of minutes it takes the plant to lose one gram of water, data which are readily transformable into other terms. The record paper is divided into seven horizontal spaces, marked by initial letters, one for each day of the week. The pen slides on the bar, which contains seven notches; and each day, when the plant is watered, the clockwork is wound, the weights are returned to the cylinder, and the pen is slipped along the bar one notch. These operations are to be performed between 8 and 9 A.M. daily, this hour being duplicated on the paper for this purpose. Each record paper therefore contains a complete record for a week. The mechanism is protected by a glass bell jar. In arranging the instument for use, one first places the record paper upon the clock-cylinder, lifting the latter vertically from the clockwork for this purpose. The paper is first wrapped tightly around the cylinder with its bottom margin matching the lower edge of the cylinder; the outer end (that marked with the letters *M*, *T*, *W*, etc.) is then lightly mucilaged on its under face and pressed tightly over the inner edge, and the cylinder is allowed to rest on a table with this part down for a few minutes, until the mucilage has set. Two or three bits of gummed paper are placed over the top of paper and cylinder to prevent any slipping of the former on the latter. The cylinder is then replaced on the clockwork, with approximately the correct time opposite the pen, and is given the exact adjustment for time by means of the central nut beneath the stage of the instrument. The pen is then filled with recording, or chronograph, ink; this may best be accomplished by aid of a pointed glass rod lifting drops from the bottle. If the pen gives too coarse a mark, it can be made finer by filling the pen with packed cotton-wool and merely moistening the wool with the ink. The pen carrier should be so adjusted that after striking the paper it springs back a trifle, and so remains until the opening of the circuit. The transpirograph is now brought so close to the scale-pan containing the plant that the outlet tube extends over a small receptacle placed on the pan to catch the weights; and plant and receptacle are then balanced exactly by weights in the usual way. The instrument and balance are then brought into electric circuit with two dry-battery cells, through the two binding screws under the transpirograph stage; and any convenient arrangement is made on the balance such that the rise of the plant-pan to its uppermost position will close the circuit, when the mechanism should operate as above described. A cut-off in the circuit is also desirable, to permit manipulation of the mechanism without affecting the record. The speed of the clockwork may be timed by the regulator under the stage. The same care must be given to this as to any other instrument of precision. The balls must not be left under conditions permitting them to rust. It is not necessary that the instrument be set immediately beside the scale-pan; it can be removed to any desired distance provided it is above the balance, the balls being made to run by gravity from one to the other through a glass tube. A special balance, having a mercury-contact circuit closer, designed for use with this instrument is among my normal apparatus and has been mentioned earlier (page 175).

METEOROLOGICAL INSTRUMENTS. Exact studies in the relations of physiological phenomena to atmospheric conditions are greatly facilitated by the existence of accurate instruments developed by meteorologists. So far as concerns our present subject, these relate to the measurement of temperature, humidity, light, and barometric pressure.

Thermometers and Thermographs. The thermometer is too well known to require comment. Very important is the registering thermometer, or thermograph. The RICHARD form (Fig. 46) has outside the case a laterally

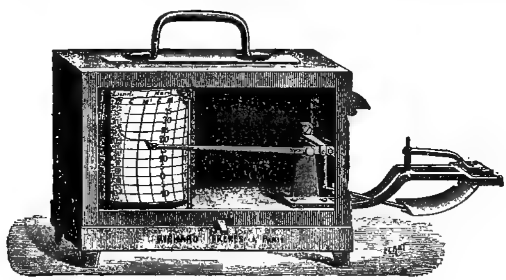


FIG. 46.—THE RICHARD THERMOGRAPH; $\times \frac{1}{8}$.
From the Price-list of Richard Frères, Paris.

flattened but lengthwise-curved thin metal tube, which is straightened by the swelling of the contained alcohol as temperature rises, and curves elastically as the alcohol shrinks with falling temperature. These movements are made to raise and lower an arm carrying a pen which writes upon a record paper ruled in degrees and hours and turned by an eight-day clockwork. Thus a continuous temperature record for a week results. The DRAPER form is shaped more like an ordinary clock, with the bulb inside and a revolving record paper in place of the face. These instruments are made also by other firms, and that of LANDER and SMITH of Canterbury, England, has the advantage, for some purposes, of recording not upon curved but upon straight lines.

Hygrometers and Hygrographs. The humidity measurer, or hygrometer, is constructed upon either of two principles. *First,* advantage is taken of the fact that certain substances, hair, horn, some plant tissues, readily absorb and release moisture from the air, and thereby alter length, or twist; pieces of these, suitably mounted and provided with a pointer moving over a graduated scale, form hygrometers. All of them, however, are variable and unstable, needing frequent standardization. One of the most useful is the MITTHOF form, sold by supply companies for about $2.00. One of the best of the hair forms is called the polymeter. Small forms of these are often called hygroscopes, which will later be described. *Second*, advantage is taken of the fact that evaporation is a cooling process, and, further, that its rapidity is inversely proportional to the moisture in the air; hence by comparing the temperature of two thermometers, one with its bulb dry and the other with its bulb exposed to evaporation from a thin water-soaked muslin covering, it is possible, by aid of tables, to calculate the exact humidity of the air. Thus is made the standard instrument, the wet- and dry-bulb thermometer, or psychrometer, the standard form of which, in this country, is the sling psychrometer of the United States Weather Bureau, obtainable from supply companies and costing about $12.00. A modification of the swinging mechanism, the cog psychrometer, has been introduced by CLEMENTS, as described in his "Plant Physiology and Ecology," 28. The registering hygrometer, or hygrograph, as constructed by RICHARD FRÈRES, has outside the case a band of hairs which lengthen or shorten as they absorb or release moisture from the air. This motion is communicated to an arm which records precisely as for the thermograph, the record paper being ruled for percentages of saturation. It gives a record of relative humidity, which is that of most importance to the physiologist. For some physiological purposes, notably transpiration, it is best to invert the curve, thus transforming it into a curve of dryness, the advantage of which is this, that it makes the condition favoring transpiration show in a rising curve as in the case of temperature and light. RICHARD FRÈRES also makes a registering psychrometer. The DRAPER hygrograph resembles outwardly the DRAPER thermograph. A new instrument, which measures the evaporation from a standard porous surface, is the vaporimeter invented by LIVINGSTON (1906, see Literature below); its application to physiological studies is illustrated by TRANSEAU in the Botanical Gazette, 45, 1908, 217.

Barometers and Barographs. These are of minor importance to the physiologist working in a laboratory, since plants are only inappreciably affected by the comparatively slight atmospheric fluctuations in one locality. Still, for some purposes they are needed, especially for making corrections in the accurate use of pressure-gauges. The most accurate instrument is the mercurial barometer, too well known to need comment. It is obtainable self-registering, but at very large expense, and the commonly used autographic form is the barograph. The RICHARD form (Fig. 47) has inside the case eight aneroid barometer boxes, *viz.*, elastic-iron vacuum boxes; these swell and shrink with variations in pressure, and communicate their motion to an arm which records precisely as for the thermograph, the record paper being ruled to correspond with millimeters of mercury of the mercurial barometer.

The RICHARD instruments are made by RICHARD FRÈRES of Paris and cost from about $35.00 to $45.00 each, without duty. They are made in substantially the same form, but at higher prices, by JULIEN P. FRIEZ of

Belfort Observatory, Baltimore, Md. The DRAPER forms are made at less cost by the DRAPER Manufacturing Company of New York City. All of these instruments are prone to work out of true and should be standardized often by comparison with standard thermometer, wet- and dry-bulb thermometers, and mercurial barometer respectively. Nor do they record with minute accuracy, though they are correct enough for most purposes.

Photometers. No form of light recorder suitable for physiological uses yet exists, for the meteorological burning-glass type and black-bulb-thermometer types record only variations between sunlight and cloudiness. CLEMENTS has described a photometer, utilizing the darkening of photographic paper ("Plant Physiology and Ecology," 73), but it is laborious of operation and does not yield a graph. Accordingly in my own laboratory I have made use of an empirical system as follows. The percentage of

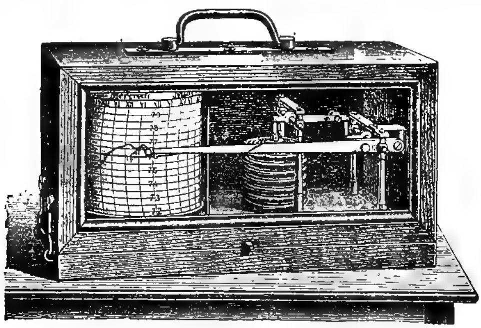


FIG. 47.—THE RICHARD BAROGRAPH; $\times\frac{1}{5}$.
From the Price-list of Richard Frères, Paris.

light of full sun at noon in this latitude, in comparison with the full light of the sun overhead taken as 1, is determined for each day of the year; then a curve for each day is constructed by drawing a parabola through the hours of sunrise, noon, and sunset, and this is the curve of full sunlight. For cloudy days this curve is lowered after the following system, *viz.*, for fleecy clouds or haze, to 90% of the full amount; for heavier clouds alternating with blue sky, 85%; for light continuous clouds, 80%; overcast dark clouds, 60%; heavy rain, 50%; heavy snow, 40%. The light record is thus drawn as a graph on the same horizontal scale as the records of thermograph, hygrograph, and transpirograph. A fuller description of the details, together with an illustration of the use of these graphs, will be found in MISS CLAPP'S paper cited under Materials earlier (page 172). The subject of light measurement, from another point of view, is treated by WIESNER in his new work, "Der Lichtgenuss der Pflanzen," Leipzig, 1908.

While the foregoing method, *viz.*, the comparison of records of contemporaneously registering instruments, solves the present problem in a general way, it does not, nevertheless, permit the exact determination of the effects of each influence singly. This can be effected only by use of a method by which a single influence can be varied at a time, leaving all the others constant. For precision studies, using potted plants, this would necessitate the use of a meteorostat, somewhat upon the plan earlier described (page 37). But using the less accurate material of cut shoots, it can be effected by use of potometers, in conjunction with a supported bell jar in which the influences can be varied one at a time.

POTOMETERS. The principle of these is this: the plant, either a shoot cut under water, a slip rooted in water, or a water-culture specimen, is sealed air-tight, by use of a bored-and-split rubber stopper and wax, into a small chamber of water, which is in communication with a small-bore, almost capillary, record tube; along this tube, which is graduated or calibrated, an air-bubble is driven by atmospheric pressure as the plant absorbs water, and the movement of this bubble may be timed. The simplicity of the mechanical problem, in conjunction with the visible effectiveness of the result, has proven a great temptation to ingenuity, and divers and protean forms have been described,—by KOHL (figured in BURGERSTEIN, 16), by SACHS (in his "Lectures," 252), by PFEFFER ("Physiology," 1, 242), by DETMER, 216 (and "Kleines Praktikum," 112), by HALL (Annals of Botany, 15, 1890, 558), by F. DARWIN (DARWIN and ACTON, 80, 83, 99), by GREEN, 89, by MACDOUGAL (Botanical Gazette, 24, 1897, 110, and "Physiology," 207, 210), by FARMER ("New Phytologist," 2, 1903, 53), by REED (Journal of Applied Microscopy, 5, 1902, 2047), by PETHYBRIDGE (Scientific Proceedings of the Royal Dublin Society, 10, 1904, 149), by OSTERHOUT, 206 (a make-shift form), by myself (first edition of this book, 80), and by several others. The requisites of a good potometer are these: (*a*) the smallest practicable capacity in order that the water may more quickly take the temperature of a new situation, and hence not vitiate the readings by volume changes; (*b*) a horizontal record tube, which prevents buoyant rise of the air-bubble, if that is used, or a varying weight of water if an air column is used; (*c*) an outer reservoir of water which can be used either to supply a reserve to the plant, or to drive back the air-bubble to the starting-point; (*d*) a calibrated as well as graduated record tube, so that the transpiration may be determined in absolute units as well as relatively. All of these merits I have incorporated in the instrument which is among my normal apparatus (page 46) and which is constructed as follows:

It has four parts. *First* (Fig. 48) is the shoot chamber, made small that its water may more quickly take a new temperature and hence not affect the record while changing volume. *Second* is the small-bore record tube, calibrated to cubic centimeters; it is bent down at its distal end, which is sealed but provided with a small lateral air-opening readily closed by a sliding piece of rubber tubing. *Third* is the reservoir connecting with the other parts through a stop-cock; it is made removable to permit use of the instrument with a supported bell jar as shown by figure 50. *Fourth* is a firm wooden base. To use the instrument one first closes the air-opening of the record tube and then fills the reservoir and shoot chamber to the rim of the latter with water boiled to expel the air. The selected shoot is now

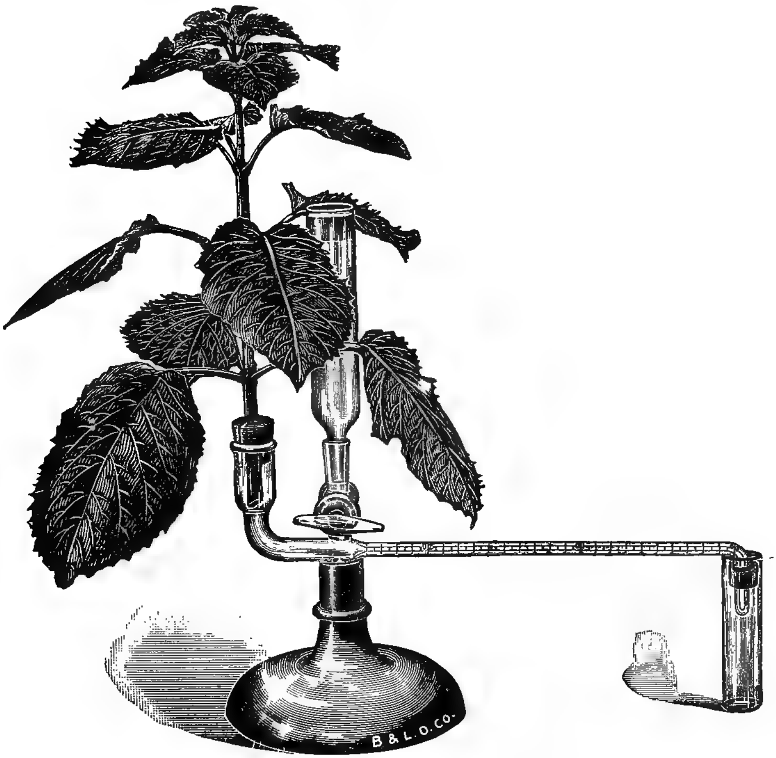


FIG. 48.—NORMAL POTOMETER; ×⅓.
Explanation in text.

cut under water and pressed sidewise into a bored-and-slit soft rubber stopper well filled with soft (stop-cock) wax; the stopper, with lower end of the shoot projecting, is now pushed into the chamber neck (ground to give a better grip) firmly enough to make a water-tight joint, but no more. The reservoir is then filled to near the top, a film of oil is added to check evaporation, the air-opening is opened to permit the record tube to fill, the stop-cock is closed, and all is ready for use. At once the transpiration makes a draft on the water so that air enters the air-opening. One may then either take the advancing end of this column as an index, or, better (since the friction of the water column in the record tube will then remain constant), may use only a bubble some 2 mm. long, which is admitted before the bent end is immersed in a vial of boiled water. When the index bubble has reached the proximal end of the tube, a cautious opening of the stop-cock will cause it to be driven back to the starting-point, and thus it is used over and over. Whilst observation is suspended, it is only necessary to close the air-opening and open the stop-cock, when the plant will be supplied from the reservoir.

A form similar in general principle, but less compact, is among the apparatus of the Stoelting Co. It is not difficult to adapt a closely similar form from a large T tube, capillary tubing, and other simple materials as illustrated in the accompanying figure (Fig. 49). The air-bubble is readily

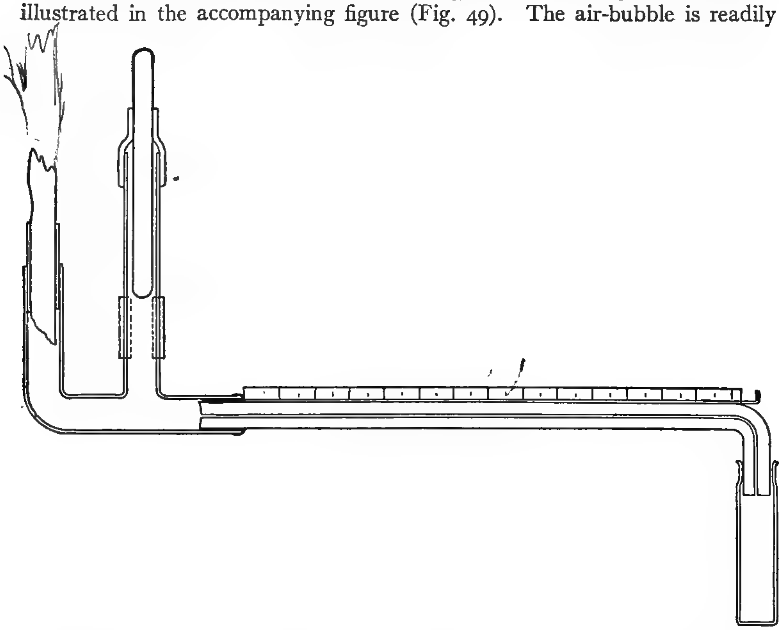


Fig. 49.—Potometer adapted from laboratory materials; $\times \frac{1}{2}$.
Heavy black is sealing-wax; the vial is suspended by wire from the hook just above it.

and accurately driven back by slightly twisting and pushing the solid glass rod in the reservoir tube, to which is attached a supporting clamp.

The potometer is quite untrustworthy as a measurer of the natural transpiration of a plant, that is, of its transpiration upon its own roots; but it is of much value for measuring, and especially for demonstrating, the relative rates of transpiration of the same plant under different external conditions. For this purpose, however, it must be used with certain precautions. The shoot selected should have some firmness of texture so as not readily to wilt, should be cut under water so as to prevent ~~clogging of the~~ ducts by air, and should be handled always as gently as possible. While results become immediately visible, these are more accurate if the shoot is first allowed to stand an hour or two in order to adjust internal pressures, etc.; and a few minutes (10–15) should be given under each new condition for adjustment in temperature and the like. While not in use the plant should be kept covered from dry air; and since the shoot steadily deteriorates, all study of it should be brought within a few hours. For comparative records one may either read the distance the index bubble travels in a given time, or else the length of time it requires to travel a given distance. The latter is the better method since it eliminates a possible error from varying diameter of the tube. One can always easily test whether the absorption shown by the record tube correctly represents the transpiration by simultaneously weighing the instrument upon a good balance.

For demonstration or other merely qualitative purposes, the external conditions may be varied by simple devices of shading, covering with a glass case, etc., the rate of movement in the record tube responding sensitively in a striking manner. Or, used by individual students, it may be carried from place to place where the conditions are different. But for exact work the external conditions must be varied under control, for which purpose the shoot should be passed into a bell jar (compare KOHL's arrangement in BURGERSTEIN, 16, and DETMER, 221). An arrangement for this purpose, supplied among my normal apparatus, is constructed thus (Fig. 50):

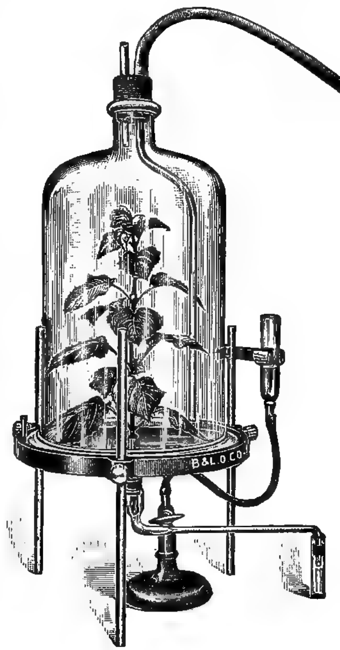


FIG. 50.—SUPPORTED BELL-JAR; $\times\frac{1}{8}$.

It consists of three parts. *First* is the bell jar of standard size and form, whose ground stopper may readily be replaced by one of rubber carrying inlet and outlet tubes. *Second* is the firm ring-support of iron having a projecting inside rim and three sockets holding metal legs which may be clamped in any desired position. *Third* are the thick glass plates which rest upon the ring; one of these is perfectly plain, for use when it is desired simply to seal in an entire plant, but the other is split through its diameter and has a hole 1 cm. in diameter in its middle. In use the stem passes through this hole, the remainder of which is completely filled by split rubber tubing and suitable wax, while the latter also makes tight the junction of the two halves of the plate and of the bell jar with the plate. If the halves of the plate tend to spread apart, they may be kept together by a small wooden wedge forced down between one of them and the iron ring.

In the bell jar, which should contain a thermometer and hygrometer, the conditions can be varied one at a time,—light by various forms of shading, humidity by drawing air through calcium-chloride tubes, or a wet sponge, into the chamber, temperature by drawing the air through a glass tube heated by a spirit-lamp or cooled by cold water or ice.

Demonstration Methods. These have been indicated above. Potometers of all degrees of accuracy and sensitivity can be adapted from simple materials, and show very striking results when the conditions are varied before a class.

The foregoing methods yield satisfactory data as to the effects of external conditions upon transpiration, but do not admit of a comparison between the different plants. For this it is necessary to expose them to precisely the same conditions, which for potted plants requires some form of meteorostat, but for shoots can be effected by potometers in bell jars. Of course the comparison must be made between equal leaf areas, preferably expressed in the gm^2h system. The same end may, however, be attained in another way by taking the means of the results given by the foregoing experiments, the various fluctuations tending to balance one another through considerable intervals of time.

The external conditions which may ~~affect transpiration~~ are, obviously, not confined to those surrounding the transpiring leaves, but must include those which bear upon the absorbing roots. Accordingly we now face this problem:

What effect is produced upon transpiration by the principal conditions affecting the water-supply?

These conditions, in order of importance, are (*a*) quantity of water available; (*b*) the temperature of the soil; (*c*) the soluble substances present.

Experiment. Prepare a plant as for the preceding experiments, and, keeping all other conditions as constant as possible, withhold all water and determine the transpiration.

Experiment. Prepare a plant as for the preceding experiment, but with a good thermometer in the soil, and for a day determine its transpiration under ordinary conditions. Then immerse the pot, nearly to the top of the metal shell or cover, in a vessel of chopped ice, and cut off all radiation, by a woolen cover, from the ice to the leaves. Stand the plant where its leaves will have the usual conditions for three hours, then remove the pot, wipe dry the cover, and weigh to determine the transpiration for that period. After leaving the plant for an hour or two to recover, replace it in a vessel containing water which is heated by a spirit-lamp until the soil is at 35°, radiation to the leaves being prevented; keep it there for three hours and determine the transpiration for that time.

Experiment. Prepare a plant as for the preceding experiments, but water it on successive days with water containing a harmless salt, *e.g.*, *n*/10 potassium nitrate (about a 1% solution), *n*/5, *n*/3, and observe the effect upon the rate of transpiration.

The student should now extend his knowledge of the quantitative phases of transpiration by a study of the subject in the literature, giving especial attention to its quantities, the effect produced upon it by external factors, its relation to evaporation; and he should express these matters in a proper exposition.

The student should turn next to a study of the transpiration structures, of which he should review and extend his knowledge until he understands, with a clearness permitting their accurate diagrammatic representation, *the construction of the leaf tissue, as to connection of vessels with the mesophyl cells, the structure of these, their connection with the intercellular spaces, the connection of the latter with the outside world, and the anatomy and mode of action of the stomata and guard cells.*

Any study of leaf structure will suggest that the stomata must play an indispensable rôle in transpiration, which presents this important problem:

What relation exists between transpiration, and the presence, number, or condition of stomata?

This may most readily be determined by applying a test of the occurrence of transpiration to leaves, the distribution, number, and open or closed condition of whose stomata is known.

Experiment. Select from among the materials described later under Stoma Quantities, leaves possessing (*a*) stomata on one side, (*b*) on both sides but in unequal numbers, (*c*) on both sides but in equal numbers, and (*d*) closed stomata (effected by partial wilting); apply to them, by some form of leaf clasp, small discs of paper impregnated with cobalt chloride dried to deep blueness over the Bunsen flame, and observe the transpiration as indicated by the reddening of the papers

Methods of Testing Occurrence of Transpiration. There are several of these, some of them crudely quantitative. Most important of all is the cobalt-chloride test whose value was first shown by Stahl (Botanische Zeitung, **52**, 1894, 118). Cobalt chloride is an inexpensive salt of a red color, very soluble in water; if discs of filter-paper are dipped into an aqueous solution of from 1 to 5% strength (the weaker giving the quicker, and the stronger the more conspicuous, test), and then are dried over a flame, they turn bright blue, returning gradually to redness on access of any moisture. The discs are most convenient for use when kept, impregnated with the salt and dried, in stoppered bottles, being given a final drying over the flame just before use; they keep good indefinitely. Of course the student will make himself familiar with the action of the material and method before applying it to the present subject. The discs may be applied to the leaf by some make-shift arrangement of pieces of mica held in place by a clamp, but can be applied much more conveniently and efficiently by some such leaf clasp as is described below. The method may be made crudely quantitative by noting the comparative times requisite for the change of color. *Second* in importance of these methods is the use of small hygroscopes, or hygrometers, contained in chambers which may be applied to the two sides of the leaf. A hygroscope is a small instrument using the hygroscopic swelling of horn, gelatin, etc., to move a pointer; an excellent form, of horn, is F. Darwin's, described in the Philosophical Transactions, **190**, 1898, 531 (figured in Burgerstein, 33); another is MacDougal's, using a celluloid and gelatin (photographers') film, described in Torreya, 1, 1901, 16, and his "Physiology," 200. Most useful of all, however, is F. Darwin's awn hygrometer, utilizing the hygroscopic twisting of *Erodium* or *Stipa* awns to carry a revolving pointer, as described in Darwin and Acton, 103, while the instrument is supplied among the Arthur apparatus (page 54). A very fair substitute can be adapted from *Erodium* awns attached by sealing-wax to corks placed inside of cut-off vials. Another form, invented by J. Aitkin, composed of a petal of an Everlasting flower with a hair, in a metal case, is mentioned in Science, **27**, 1908, 475. As a rule it is not necessary to seal the chamber to the leaves, but this can be done either by vaseline-wax or by strips of thin rubber sheeting bound around, and projecting beyond, the rim of the chamber. *Third* of these methods is the use of weighed water-absorbing chemicals in the chambers on the two sides of the leaf, obviously a quantitative method, introduced by Garreau and figured by Detmer, 215. *Fourth* is another quantitative method, depending upon loss of weight with stomata variously closed in severed leaves, as described by Darwin and Acton, 102. *Fifth* is the method recently described by F. Darwin in the Botanical Gazette, **37**, 1904, 81, in which the cooling effect of transpiration is utilized, by employing two small platinum resistance thermometers on opposite sides of the leaf, recording upon a drum. *Sixth*, there is a simple method, of some value for demonstration, in which watch-crystals are applied to the two surfaces of the leaf, being sealed on if the leaf is rough, the collection of moisture on the glass giving some idea as to the transpiration.

LEAF-CLASP CHAMBERS. For applying the cobalt-chloride discs to the leaf while protecting them from the moisture of the air, STAHL used little sheets of mica held in place by clamps; and where the leaf was very rough, he sealed the edges by a special wax. An excellent modification of this, using glass slips, is given by LINSBAUER, 38. For greater accuracy and con venience of application of this test, as well as for some allied uses, I have devised a special leaf clasp originally described in the Botanical Gazette, **39**, 1905, 148, which is among my normal apparatus (page 46) and constructed as follows:

Two similar brass rings (Fig. 51), "chamber rings," each 30 mm. in diameter and 3 mm. in depth, are attached at the ends of parallel flexible-elastic bars, so arranged that they hold the rings firmly and exactly edge to edge, while allowing of their separation, by means of a screw, to any desired extent. A second screw permits of their tighter closing, while manipula-

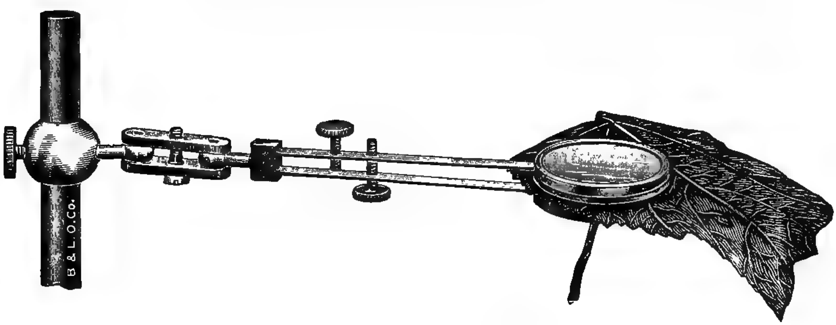


FIG. 51.—LEAF CLASP; $\times\frac{3}{5}$.

tion of the two screws together allows a certain amount of adjustment of the rings to fit surfaces not in the same plane. For each chamber ring there are provided two accessory rings. One of these is right-angled in section and holds a removable cover-glass, so that when pushed over the outer edge of the chamber ring, it converts the latter into a glass-topped chamber, as shown in the figure. If discs of filter-paper treated with cobalt chloride are so placed in the chambers as to rest against the leaf, their change of color in transpiration may be observed with the greatest clearness and facility. Incidentally the tightness of the chambers (when not on the leaf) permits the papers to retain their dryness and blue color for a considerable time, so there is no need of haste in applying them to the plant. The second accessory ring is broken, and is intended to hold paper, rubber, or other fabric tightly to the chamber ring either inside or outside thereof. Thus if projecting veins prevent a connection of chamber with leaf sufficiently tight for some special purposes, a band of thin rubber may be held by the broken ring in such a way that it will project against the leaf, filling the spaces between the veins. The discs can be so held in place, by flaps under the broken rings inside the chamber, that they need not thereafter be removed, being dried in place. The universal joint attaching the clasp to a support permits it to be set in any desired position. An addition of small mirrors set at 45°, thus permitting both sides of the leaf to be seen at once, would make a desirable addition for demonstration, and even for exact study.

STOMA QUANTITIES. The most extensive study of stoma numbers, sizes, and distribution yet made is that by WEISS in "Jahrbücher für wissenschaftliche Botanik," 4, 1865–66, 123, 196, and from his tables there are selections in DETMER, 170, and especially in GOODALE, 71. For our common greenhouse plants a comprehensive study has been made in my laboratory by MISS SOPHIA ECKERSON, with results later to appear, probably in the Botanical Gazette, Vol. 46. In synopsis they are as follows:

Distribution. Only about two-fifths of our common greenhouse plants have any stomata upon their upper surfaces, and then almost invariably in far smaller number than upon the lower. Those having them upon both surfaces are, in order of nearness to equality of the two surfaces, Wheat, Oats, Indian Corn, Windsor Bean, Horse Bean, Sunflower, Marguerite, Ivy Geranium, Castor Bean. Only floating leaves of water-plants have stomata upon the upper surface alone. The commonest plants having them upon the lower surface only are *Abutilon*, *Coleus*, Poinsettia, *Ficus*, Fuchsia, English Ivy, Heliotrope, Oxalis, *Primula*, *Salvia*, *Senecio*, *Tradescantia*, *Tropæolum*.

Numbers. The most numerous stomata occur, in order of abundance, on the under surfaces of *Abutilon*, *Ficus repens*, String Bean, Squash, *Salvia involucrata*. The fewest occur in general upon those having the largest.

Size. The largest stomata, in order of size, occur on the under surfaces of Wheat, Tulip, Oats, *Primula sinensis*, Marguerite, and Wandering Jew. There is in general an inverse proportion between number and size.

Constants. Taking all the 37 plants of this study collectively, the number ranges from 0 through a mean of 121 to 484 per square millimeter, whence we may derive a conventional constant of 0–100–500 mm^2, or a mean of over 100 millions to the square meter. The mean size of the open pore is 17.7×6.7 (conventionally 18×6) microns, and the mean area is 92 (conventionally 100) square microns. The total pore area for a square millimeter is therefore 11,132 (conventionally 10,000) square microns, which means that when all the pores are open about one-ninetieth (conventionally one-hundredth) of the epidermal surface is open.

A very important question here arises as to whether transpiration is regulated, directly or indirectly, by purely physical causes, or whether the regulation is in part at least vital or physiological. This subject has recently been studied by LIVINGSTON through his new method of relative transpiration, *viz.*, comparison of transpiration with evaporation from a standard water-surface, and by LLOYD through the physiology of stomata.

With their results the student should make acquaintance through their papers cited under Literature below.

Another matter of new interest is a connection which has been found to exist between transpiration and growth, a connection so close as to make it possible, in certain cases, to use the one as a measure of the other. The subject is discussed by LIVINGSTON in the Botanical Gazette, **40**, 1905, 178.

The data the student has now accumulated will lead him to an inquiry into the exact significance of transpiration to the plant, whether it is a process of physiological value in itself, or is merely incidental to other functions. For this he must make himself acquainted with the present state of our knowledge of *the energy relations of transpiration, its possible connection with the lifting of the water threads in the ducts, and in how far it is purely physical and how far physiological.* And he should note in what way the structures concerned in its performance are related to those concerned in photosynthesis and respiration. And he should give a clear exposition of this important subject.

The copiousness of transpiration in conjunction with its extreme sensitiveness to both atmospheric and soil conditions makes the process the basis of very important ecological phenomena; these the student should now work out and express, after the same general plan which he has followed under photosynthesis (page 113).

TRANSPIRATION QUANTITIES. These for forest and field plants are summarized by PFEFFER, 1, 250. For greenhouse plants, used in educational work, they are given very fully in MISS CLAPP'S paper cited earlier under Materials, a paper which also gives graphs expressive of the relations of their transpiration to external conditions. She shows that the transpiration of greenhouse plants in general has a mean of 48.73 grams per square meter per hour during the day and 8.9 during the night, whence may be derived a conventional constant of under 50 gm^2h for day and under 10 gm^2h for night. Her figures also demonstrate a range, taking day and night together, from 1.12 through a mean of 28.81 to 257 grams per square meter per hour, which may be expressed conventionally as 1–30–250 gm^2h.

LITERATURE OF TRANSPIRATION. In addition to the invaluable works of PFEFFER and of JOST, there is a very important and satisfactory monograph upon Transpiration, "Die Transpiration der

Pflanzen," by DR. ALFRED BURGERSTEIN (Jena, 1904), a type of work of which we need many more. On the energy relations of Transpiration, the most important work is by BROWN, described in two papers, in Nature, **60**, 1899, 479, in Proceedings of the Royal Society, **76**, 1905, 29 (review in Botanisches Centralblatt), and in Nature, **71**, 1905, 522. Transpiration being bound up with Transport, there is important matter in DIXON'S papers cited under that subject earlier. Important new papers are LIVINGSTON'S "The Relation of Desert Plants to Soil Moisture and to Evaporation," Carnegie Institution, 1906, and LLOYD'S "The Physiology of Stomata," Carnegie Institution, 1908.

(*b*) *Guttation.*

The multiformity of conditions of transpiration will sooner or later suggest to the student the query whether water is ever eliminated by the plant in other than vapor form. Casting about for evidence upon the subject from experience, he will recall the familiar garden phenomenon shown by leaves of Canna and some other garden plants on cool spring evenings following hot days, when these plants show streams of water flowing down the leaves. Recalling now the conditions under which this occurs, it is evident that it accompanies a transition from highly favorable to unfavorable conditions of transpiration. The matter may be experimentally tested, involving the following problem:

Is liquid water released by young plants when favorable are followed by unfavorable transpiration conditions?

EXPERIMENT. Select a young *Tropæolum* (Garden Nasturtium) or a pot of young Oats (or other grass plants), and supply for two or three hours the best conditions for transpiration, that is, warmth, bright light, and ample water. Then suddenly give the reverse of these conditions, which is conveniently done by covering with a darkened bell jar over which a stream of cold water may advantageously be run. Observe any new appearances of the leaves.

The student should now inform himself as to the extent and activity of guttation in plants, its relation to much of the "dew" of our native plants, the function of hydathodes, and the exact physical conditions and significance to the plant of the process. Also he should consider the relation of the formation of ice crystals in the "Ice Plants."

(*c*) *Excretion.*

The elimination of waste materials from the plant, whether these are by-products of various phases of metabolism, or materials absorbed into the plant incidentally along with useful materials, has from the present point of view three phases,—the excretion of gases, of minerals, and of plastic or liquid substances.

Excretion of Gases.

This is a matter of considerable importance owing to the fact that oxygen is a waste product in photosynthesis and carbon dioxide in respiration. Yet the physics of the subject, in the light of the foregoing studies, is very simple. The gases diffuse from their places of release through the water of the protoplasm to the surfaces of the cell walls, and thence diffuse from solution into the intercellular system, and thence outward through the stomata, the energy being supplied by heat from the surroundings.

Excretion of Minerals.

Of mineral matters useless to the plant there are considerable quantities, as to the nature of which the student should inform himself. But plants have developed no system for getting rid of them, and either store them up by a process physically the same as Secretion, in spare tissue, or else drop them with bark, leaves, and other deciduous parts. The student should inquire as to the known or supposed extent to which plants actually transport substances into parts about to fall.

Excretion of Plastic or Liquid Matters.

This subject is almost wholly concerned with the possible excretion of organic substances by roots, the possible formation of acids by these belonging under Secretion, where it has already been noted. The excretion of organic matters by roots, which may ultimately prove a matter of high ecological importance, has been most fully studied at the Bureau of Soils of the United States Department of Agriculture, and may be found discussed in their Bulletins, Nos. 28, 36, and 40, and by SCHREINER and REED in the Bulletin of the Torrey Botanical Club, **34**, 1907, 279. A note by FLETCHER in Nature, **76**, 1907, 518, and another in the same journal, **69**, 1903, 162, should also be consulted.

SECTION 2. THE PROCESSES OF INCREASE.

We have now considered the making of its food by the plant and the use thereof, with the modes of absorption, transport, and removal of the various substances involved. All of these processes, however, are concerned simply with the maintenance of the life of the individual, and are without particular reference either to the increase of those individuals or their adjustments to their surroundings. We come now to consider the processes of increase, and it is evident that they fall into two, according as they are concerned with:

1. The increase of individuals in size, or *Growth*.
2. The increase of individuals in number, or *Reproduction*.

9. GROWTH.

Observation of the growth of individuals soon shows that this process includes two phases quite different in nature, which are:

(*a*) Enlargement in bulk of parts already formed, or *Auxesis*.
(*b*) Formation of new parts, or *Differentiation*.

(*a*) *Auxesis*.

Turning to enlargement, or auxesis, and seeking a basis for its exact study, it becomes evident that, as the higher plant is built up by the repetition of a large number of a few kinds of organs, notably leaf, root, and stem, we must study the enlargement of each of these. The first step in this inquiry will naturally have reference to whether each of these organs swells uniformly throughout, or more rapidly in some parts than others, thus presenting to the student the definite inquiry:

Do growing leaves, roots, and stems enlarge uniformly throughout, or more rapidly in some particular parts?

This may be determined by covering the organs while young with evenly spaced indelible marks, and noting the alterations in spacing as growth proceeds.

Experiment. (*a*) Select a plant with rapidly growing leaves, and mark some of the younger of these into equal areas of known size, say 2-mm. squares. Place under favorable conditions for growth, and, when grown, compare the spread of the marks with the original spacing.

(*b*) Using either the same plant, or another with rapidly growing, slender, nearly naked stem, mark this while young by evenly spaced lines, say 2 mm. apart; place under conditions favorable for growth, and later note the spread of the marks.

(*c*) Germinate some strong seeds (Horse Beans, Corn, Peas) in sphagnum; when the roots are about 2 cm. long, lay one flat on the damp moss and mark it from the tip backwards with evenly spaced marks, say 2 mm. apart. Arrange with a clean thistle-tube so that the seed is packed with wet moss in the bulb, and the root is in the tube (Fig. 52), which is slightly inclined from the vertical to bring the marks upward, so they will not rub off. (*Or* an equivalent from glass tubing or from two glass plates may be arranged.) Place under conditions favorable for growth, and note the spread of the marks.

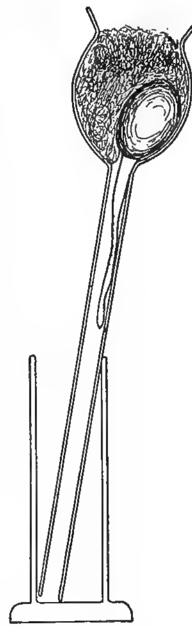


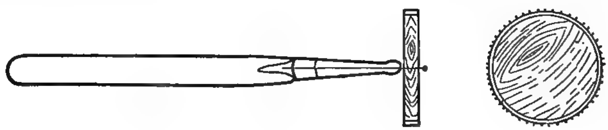
Fig. 52. — Arrangement for study of growth of roots; $\times\frac{1}{2}$.

The bulb is packed with sphagnum moss.

Space-markers. Various simple arrangements may be improvised, such as laying the part on a moist surface close beside a ruler and making the marks with a fine brush, a pen, or a stretched thread dipped in waterproof India ink. *Or* the leaves may be punched by pins set through a flat cork in squares 2 mm. apart But very much better for efficiency and convenience are rubber-stamp markers, of which two forms are to be supplied among my normal apparatus. For roots and stems the marker is a wheel (Fig. 53) with its rim of cross lines 2 mm. apart; it turns freely on a handle and may be rolled along the part, marking it perfectly in a moment. For leaves the marker is a disc ruled in 2-mm. squares which may be pressed by spring or scissors-like handles (Fig. 54) against the leaf, which is held on another disc provided with a slot for the petiole and made soft by a layer of felt.

The stamps are inked on a sheet of glass, on which waterproof India ink has been thinly and freshly spread. Care must be taken in marking the roots that these are not long exposed to the dry air of the room.

The foregoing experiments will show very clearly where growth is most active in typical leaves, roots, and stems. From this basis the student should extend his knowledge, by additional experiments upon other organs if practicable, and by study of books in any case, until he gains so clear an idea of the distribution of growth that he can *construct a diagram to show its distribution and relative intensities throughout a typical complete higher plant.*



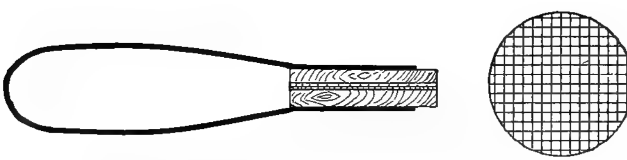


FIG. 53 (UPPER). SPACE-MARKING WHEEL; $\times \frac{2}{3}$.
The rim is a rubber stamp marked with plain cross lines 2 mm. apart.

FIG. 54 (LOWER). SPACE-MARKING DISC; $\times \frac{2}{3}$.
It consists of a rubber stamp marked in squares 2 mm. on a side, connected by a spring handle with a felt-covered disc.

It is essential next to determine how rapid this growth may be, which presents the experimental problem:

What is the rate of growth of leaves, roots, and stems under ordinary conditions?

This may be determined by direct measurements, at definite time-intervals, with fine rulers or calipers; but most growth is so slow that in practice it is advantageous to make use of magnifying arrangements, which may be arranged not only to exhibit growth (auxoscopes), but also to measure (auxanometers), and even to record it (auxographs). In any case it is best to begin the study with a structure of rapid growth and easy manipulation.

EXPERIMENT. Select a stem of rapid vertical growth (*e.g.*, a flower-stalk of Grape Hyacinth or other bulbous plant); when it has just appeared above ground, arrange it for study by aid of an auxanometer, or, better, by an auxograph; give favorable conditions for growth, and secure a record through its entire cycle. The recording meteorological instruments should stand in the vicinity.

PRECAUTIONS. Aside from a sufficient temperature, good light, and freedom from injurious gases, the most important condition of growth concerns proper watering. The air must be kept at least moderately humid, by copious sprinkling of the experiment house, and the pot must not be allowed to dry out excessively, which may be prevented by enclosing it in a larger one, with wet sphagnum moss between. It is even advantageous to surround the plant under experiment by a thicket of other plants. Since watering causes a swelling of the soil, and hence a slight lifting of the entire plant, with a consequent error in the growth record, the watering should be done either once a day when the readjustment of the instrument is made, or, better, should be effected by a hydrostatic or self-regulating device (described in Part III), of which LIVINGSTON'S should be by far the best for this purpose. *Or*, in some special cases, it may be possible so to enclose the plant in a saturated atmosphere that a single thorough watering at the start will suffice to the end of the experiment.

AUXOSCOPES, AUXANOMETERS, AND AUXOGRAPHS. Of these there are many forms which may be classified as follows. Auxoscopes are of subordinate importance, and will be considered under Demonstration Methods below.

Auxanometers. Of these there are several types, applicable to different phases of growth as follows: *First*, and most exact, is the *horizontal microscope*. It stands on a firm tripod base, can be levelled, is adjustable for height, has an objective of long focus with an ocular micrometer, and is provided either with a vernier scale or a screw micrometer. In use it is kept permanently beside the growing object, whose increase in height is read in part on the ocular scale and in part on the vernier. Owing to the interference of leaves, with their sundry nutations, etc., it is better adapted for use with small leafless structures, such as molds and roots. Its use for growth measurement seems to have been introduced by SACHS in 1872; it was improved by PFEFFER, whose form is obtainable from ALBRECHT of Tübingen. WIESNER has also a form (Zeitschrift für Mikroskopie, 1893, 147), as has F. DARWIN (DARWIN and ACTON, 153), the latter obtainable from the Cambridge Scientific Instrument Company. A form by W. WILSON of London permits both vertical and horizontal measurements. An excellent instrument designed by BARNES is sold by the BAUSCH & LOMB Optical Company; another is offered by LEITZ. A Cathetometer, used in Physics, is also admirable for this purpose, as SACHS pointed out. An adapted arrangement, utilizing two ordinary microscopes, has been described by RICHARDS (Torreya, 3, 1903, 136). *Second*, there is the *cup micrometer*, invented by F. DARWIN (DARWIN and ACTON, 150), and supplied by the Cambridge Scientific Instrument Company. A needle, attached by a silk thread passing over a pulley to the tip of the plant, makes an easily seen contact with a surface of oil or mercury in a cup adjustable for height by a micrometer screw. But the instrument appears to have no special merits for ordinary use. It is in reality a refinement of SACHS' early arrangement in which a pointer, attached to a weight on a thread passing from the plant over a pulley-wheel, descended along a scale. A crude form of this is given by DARWIN and ACTON, 150. *Third*, there is the arc-pointer, invented by SACHS (Lehrbuch, 2d edition, 1870, 632); in this a weighted thread attached to the tip of the plant passes over a pulley-wheel carrying a light pointer which moves over a graduated arc. This form is most effective for educational use, being especially valuable for lecture demonstrations. A good form of it is supplied by ALBRECHT, and another by the STOELTING Company, while a simple make-shift form was pictured by BESSEY in his "Essentials of Botany" (Holt & Co., 1896), 89. Another is given by MACDOUGAL in his "Elementary Plant Physiology," 18, though his OELS form, 19, exhibits a too heavy turning weight. A modification of this, in which the pointer is replaced by a ray of light reflected from a mirror carried on the pulley-wheel, or an arm thereof, has also been used (MACDOUGAL, op. cit., 21). *Fourth*, there is the elaborate balanced crescograph of BOSE, described in his book "Plant Response" (London, Longmans, Green, & Co., 1906), an instrument the elaboration of whose combination of levers, mirrors, floats, and hand-recording cylinder seems to preclude the possibility of accuracy.

Auxographs (*Autographic, or Recording, Auxanometers*). Of these many forms have been devised. Of precision forms the first was invented by SACHS about 1872 ("Gesammelte Abhandlungen," 691, figured in his "Lectures," 557). It consisted of a large vertical excentrically placed cylinder turned by a weight-and-pendulum clock, and having on one side a smoked paper, which was scratched by a pointer carried on a wheel connected with the plant by a thread. A defect in the record was introduced by the arc, instead of the vertical, movement of the pointer. This was overcome in WIESNER'S instrument (Flora, 1876, 467, figured in his "Elemente der wissenschaftlichen Botanik," 3d ed., 1, 269), in which the pointer was replaced by a large wheel which permitted the vertical drop of a weighted marker suspended by a thread from its circumference, and he also used a compact spring clock. Some improvement in compactness, and in simplifying the mode of guiding the pointer, was made by PFEFFER (whose instrument is pictured in DETMER, 378, and a simple form on the same principle is figured by NOLL in the Bonn Text-book). These instruments all made the record upon a continuously revolving cylinder, but BARANETZKY (in his "Die tägliche Periodicität der Langenwachsthum," 1879, 21, figured in VINES' "Lectures," 399) introduced a fixed cylinder moved horizontally at regular intervals by an electrically released clockwork, thus making the record appear as a series of descending steps. PFEFFER improved BARANETZKY'S instrument in details, and produced the form now regarded as the standard autographic auxanometer. It is figured in PFEFFER'S "Physiology," 2, 21, and is supplied by ALBRECHT of Tübingen. Other accurate forms have also been constructed. A form using the "occasional release" principle, but with a return to the old arc-marking pointer, is F. DARWIN'S form, described and figured in DARWIN and ACTON, 154, and supplied by the Cambridge Scientific Instrument Company. Another form of great exactness was invented by FROST (Minnesota Botanical Studies, No. XVII, 1894); the thread from the plant is carried over a toothed wheel so arranged that it closes an electric circuit after definite amounts of growth, thus marking a continuous record upon a revolving cylinder which may be removed to any desired distance. Another, in which a descending pointer marks on blackened rods carried before it by clockwork, is described by GOLDEN and ARTHUR (Botanical Gazette, 22, 1896, 463). Another, in which an arc-marking pointer records on a continuously turning cylinder, is that of CORBETT (Ninth and Twelfth Reports of the West Virginia Experiment Station, 1900). Another, substantially similar in principle to the last, but utilizing the levers and clockwork of a Richard thermograph, is described by MACDOUGAL, 291. Still another form, on a photographic principle, has been invented by KOHL (Berichte der deutschen botanischen Gesellschaft, 20, 1902, 210); in this the growth of the plant permits the descent of a slide, carrying a tiny electric light in front of a minute opening, down the front of a box which contains a revolving cylinder covered with photographic paper. But this instrument would seem liable to serious practical errors. I have myself devised a precision form, working upon the same general principle as FROST'S, but eliminating the threads; and this will later be supplied among my normal apparatus. It will have the advantage that the part in connection with the plant will not exceed a watch in size, and will be practically water-proof, while the recording cylinder can be placed at any distance. Also I have devised a simpler student form, in which a paper carried on the rim of a magnifying wheel revolved in the usual way is marked each hour by the hand of a cheap watch.

Adapted auxographs in many forms have been described, especially in this country: one recording upon a smoked-glass plate carried on a movable carriage released at intervals by clockwork, by BUMPUS (Botanical Gazette, 12, 1887, 149); one recording upon a cylinder released at intervals by an electrical device, by BARNES (Botanical Gazette, 12, 1887, 150); one utilizing a small hanging balance to carry a pointer recording on a blackened arm moved by a simple clock, by STONE (Botanical Gazette, 17, 1892, 105); one where a hanging tin cylinder on which a pointer is recording is moved slightly once an hour by the hand of a clock, by F. DARWIN (DARWIN and ACTON, 1905, 156); one in which a glass pen hanging from the rim of a magnifying wheel turned by growth of the plant marks on a cylinder revolved by a simple clock, by myself (Botanical Gazette, 27, 1899, 260, and in a much improved form in first edition of this book, 103): and it is this instrument, with a change in the clock, which is supplied by the Cambridge Botanical Supply Co.; one in which a pointer connected with the plant marks on a vertical blackened strip moved once an hour by a simple clock, by LLOYD (Torreya, 3, 1903, 97, and School Science, 1903), forming one of the best of the simple instruments; one very similar to the latter in principle, but somewhat more elaborate, by SCHOUTEN in Flora, **97**, 1907, 116. I have myself constructed a later form, an improvement over that described in the first edition of this book. It has proved very satisfactory, especially for class demonstration, since its principle is so obvious, and the results can be seen from a considerable distance. Its construction is shown by the accompanying figure (Fig. 55). A wooden base, with handles for transport and screws for levelling, carries an attached framework which supports the magnifying wheel, recording cylinder, and clockwork, the latter being fastened to a tripod high enough to allow it to be wound and regulated from beneath. The clock is a Waterbury alarmless (costing one dollar), with all surplus parts removed, leaving the steel spindle, which turns once an hour, projecting above the works; these are protected from dust by tight-fitting cardboard pieces resting on the frame of the works. The record cylinder, 2.5×30 cm., is turned from hard wood, with a hole at one end for the spindle (a small pin projecting down between the cogs on the spindle will make it revolve with the latter) and a hole at the other for a pin to hold it upright. The cylinder is covered with paper—preferably cross-section paper ruled in millimeters—put on tightly and gummed by one edge, which overlaps the other in a direction such that the pen will not catch upon it. The magnifying wheel contains in one piece four concentric wheels, respectively 1.5, 3, 6, 12 cm. in diameter (to bottoms of their grooved rims), and a small hole through the axis allows it to be supported by a pin held in a needle-holder. It should turn freely, come to rest in any position, and be coated lightly with shellac to prevent warping. There should be provided a spring clamp to hold it still while the experiment is being started. The pen is made from small glass tubing drawn to a capillary point and bent at right angles, as shown by the figure, the point being smoothed by rubbing gently on a piece of ground glass. It is filled with chronograph ink (drawn into it by suction), and supported in a holder made from a brass paper-fastener. This holder has a small hole through which passes a guide-wire running from the frame above to the works below, thus preventing the pen from becoming jarred away from the cylinder. The combined weight of pen and holder should be just great enough to turn the magnifying wheel with certainty. The plant being placed in position with the wheel clamped, a thoroughly waxed fine silk thread is tied in a loose loop just under the tip of the stem, is run once around the smallest wheel (for greatest magnification), and fastened in a nick in the wood. Another waxed thread is then attached to the penholder, is run over a small pulley-wheel on the frame, is turned twice around the largest wheel, and is fastened. The whole arrangement is such that when the wheel-clamp is released the pen will start at the top of the cylinder and descend as the plant grows, tracing a record, which, owing to the hourly revolution of the cylinder, will be a spiral line crossing any given vertical line once an hour. A lesser magnification is given, of course, by use of the other wheels. It is best to have two cylinders, so that a fresh record paper may be substituted without delay. The record papers may be preserved either as cylinders, or, better, flattened and attached to a board, in which latter case it is easy to rule lines to show not only the hourly, but even lesser periods of growth. This apparatus has, incidentally, the advantage that it may be applied also to other measurements involving rise or fall of an object, of a water-level, etc.

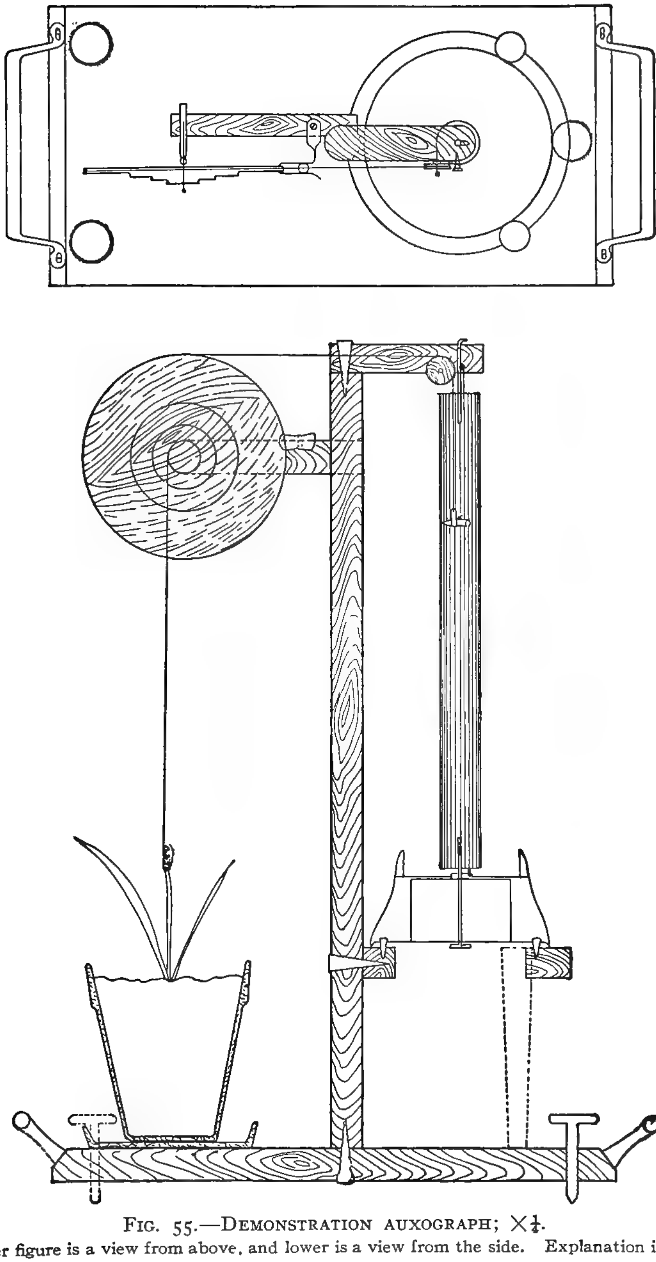


FIG. 55.—DEMONSTRATION AUXOGRAPH; $\times \frac{1}{4}$.
Upper figure is a view from above, and lower is a view from the side. Explanation in text.

A special form of adapted auxograph for recording growth in length of roots is described by STONE in Botanical Gazette, **22**, 1896, 258.

All, practically, of the existent auxographs require the use of threads for connecting plant and instrument; and in this lies their greatest source of error. For no thread has yet been found which does not alter its length with humidity changes of the atmosphere. In my laboratory I have had repeated efforts made to find such a thread. Waterproofing, either by coating, soaking, or boiling the thread with various waxes, liquid rubber, and oils, does not succeed, or only at the expense of making the threads too stiff to work properly over the wheels. Substitutes of wire, fine chains, and quartz fibers are all impracticable for one reason or another. Threads combined from material of opposite humidity relations suggested by PFEFFER do not work, for the reason that the materials have no stiffness and hence the shortening strands carry the lengthening strand passively with them. So marked is the hygroscopic shortening of some threads, that an apparent marked fall in the rate of growth registered by an auxograph may be due in reality to shortening of the thread in the drying air, and sometimes this error may become so great as to actually make it appear that the plant is shortening. Upon the whole the most practicable thread is of fine twisted (not untwisted) silk, thoroughly waxed with beeswax well rubbed in, and kept just as short as possible; this applies especially to the one connecting with the plant, for any alteration of this one is of course magnified on the record. Then the plant should also be given as uniform humidity conditions as possible, including, when practicable, a saturated atmosphere.

All of the above-described instruments were designed for measurement of growth in length, though some of them are, incidentally, equally applicable to growth in thickness. Of special arrangements for the latter, the more important are these. Of non-recording forms, the best are undoubtedly magnifying calipers, as used by JOST (Berichte der deutschen botanischen Gesellschaft, **10**, 1892, 600); some form of micrometer (the ZEISS micrometer) was used by EWART (Annales du Jardin Botanique de Buitenzorg, **15**, 1898, 188). A modification of the latter was used by F. DARWIN, where he made the micrometer carry a point into contact with a dish of oil placed upon the thickening part (Annals of Botany, **4**, 1889, 118, and **7**, 1893, 468). Of recording forms, MACMILLAN used a movable wooden jacket in connection with the BARANETZKY auxograph (Botanical Gazette, **16**, 1891, 149, and American Naturalist, **25**, 1891, 462), and KATHERINE GOLDEN has described an arrangement whereby a magnifying glass rod, recording on a revolving drum, is pushed laterally by a growing stem held in a Y-shaped support (Botanical Gazette, **19**, 1894, 113).

DEMONSTRATION METHODS. For general student use I have found nothing so effective as the demonstration auxograph above described, recording the growth in length of a flower-stalk of Grape Hyacinth. For demonstration before an audience, and to show growth actually in progress, a good auxoscope could no doubt be made from the arc-pointer of SACHS, with a very long arm, or, and better, from a mirror arranged to reflect a long beam of light when moved by the growth of the plant. Auxoscopic arrangements for projection upon a screen are described by KOHL, Berichte der deutschen botanischen Gesellschaft, 20, 1902, 208, and by PFEFFER in a paper earlier cited (page 23).

The preceding experiment will yield an accurate record, which should be transferred to a graph, of the increase of a stem in length. The same apparatus may also be applied to growing leaves and roots; and if the student has time and desires to follow this subject, he will be aided by the following suggestions:

SUGGESTED EXPERIMENTS. (*a*) Prepare a flower-pot moist-chamber, as described later under Geotropism, and fasten to a cork near its top a seed (*e.g.*, Horse Bean) with a strong-growing root. Place over the root a small sealed glass tube 5 mm. deep, which is connected by threads, through the hole in the pot, with an auxograph. Or the arrangement described by STONE in Botanical Gazette (22, 1896, 258) may be used. (*b*) Arrange a growing leaf so that it is supported in a horizontal position, but is free to expand; attach threads, by shellac, on its tip and one margin, and carry these out horizontally to auxograph wheels.

A striking feature of the auxographic records is the great fluctuations they show in the rate of growth, and it is natural to infer that these fluctuations are probably due to changes in the variable external physical conditions. Obviously this is a matter of consequence, requiring a definite experimental study, and we consider first the most important of the external conditions, and ask:

What effect is produced upon growth by temperature?

This may be tested by either of three methods: *first*, by a comparison of the growth and temperature graphs of the preceding experiment at times when the other conditions are fairly constant, or, *second*, by conducting an experiment similar to the preceding in a meteorostat (page 37), where temperature may be varied while the other conditions are kept constant, or, *third*, by exposing a series of similar plants to various degrees of temperature, the other conditions being kept the same for all, an end which can be accomplished by use of thermostats.

EXPERIMENT. Prepare 10 two-inch pots with seedling mixture and sow, exactly alike in each, 10 similar Oats. Place the pots in the chambers of a differential thermostat kept heated from 5°–60°. Supply uniform light, daily aeration, and sufficient water to each. When the tallest seedlings reach the top of their chamber, close the experiment, measure the average height of the plants in each set, and plot a graph of the results.

THERMOSTATS. Of these, instruments for maintaining a constant temperature regardless of external variations, many forms, from large rooms down to small ovens, and of all degrees of precision, have been developed, especially in recent years for bacteriological purposes. There is a firm in Berlin, HERMANN ROHRBECK, making a specialty of their manufacture. Forms constructed especially for certain botanical purposes have been described by PFEFFER (Berichte der deutschen botanischen Gesellschaft, 13, 1895, 49) and by JOST (Botanische Zeitung, 55, 1897, 25). But all of the above mentioned are dark, or at least lighted only from one side. The first lighted form was that of SACHS (Jahrbücher für wissenschaftliche Botanik, 2, 1860, 338), consisting essentially of a metal box, with a double water-filled wall, fitting outside a flower-pot which is covered by a bell jar, the whole heated by a lamp below; and this, modified in details, is figured in his "Lectures," 278, and with the addition of a regulator in PFEFFER'S "Physiology," 2, 83, though the proximity of gas-fumes in this form might well be a great injury. This instrument is of course a uniform thermostat giving only a single temperature, and is usable differentially only by employing several at different temperatures, or (less satisfactorily) by changing its temperature from time to time.

Differential Thermostats, viz., those giving a series of temperatures, and these alterable at will, have been constructed for special purposes. There is one made by LAUTENSCHLAGER of Berlin, and another by PAUL ALTMANN of Berlin. The latter has a series of ten chambers, cooled by ice at one end and warmed by water-pipes and a gas-jet at the other. But the chambers are dark and the instrument is very costly. I have, however, myself devised, and am using, a much simpler and lighted instrument, which will later be obtainable among my normal apparatus (Fig. 56). It consists of a copper trough separated, by removable partitions, into ten compartments, covered by glass boxes; it is heated at one end from a copper box warmed by gas controlled by a regulator, and is cooled at the other end from a copper box in which cold water is kept circulating. A proper arrangement of pipes conducts the gas-fumes completely away from the vicinity. The temperature of the chambers grades evenly from end to end, and may be made to differ any desired amount. A substitute could be improvised no doubt, especially if fewer chambers are used, from a bar of metal resting at one end upon steam-pipes, and cooled by running water or a box of ice at the other end, the pots being covered by large tumblers or bottles. A make-shift method, often employed in elementary work, consists simply in placing the pots in different positions known to differ in temperature, but this is so liable to introduce differences in other conditions also as to be very unreliable.

Where it is impracticable to run the instrument overnight, it should be placed in the coolest available safe place in order to check the growth uniformly throughout.

The graph of growth given by the earlier experiment shows not only the fluctuations we are now studying, but also a remarkable general curve, illustrating *the grand period of growth*, a matter of much importance on which the student should now inform himself by reading. Further, the form of this graph suggests the form of another which the student obtained by his study of protoplasmic streaming, and he should inquire whether there is any relation, or only an accidental resemblance, between these two. And other graphs from other experiments will show a resemblance to these, which needs explanation. Also, the student should here recall and observe familiar phenomena in Nature as to the relations of growth to temperature, and should correlate them with the above experimental results. And he should consider also the resistance of plants to very high and very low temperatures, with its probable physiological basis.

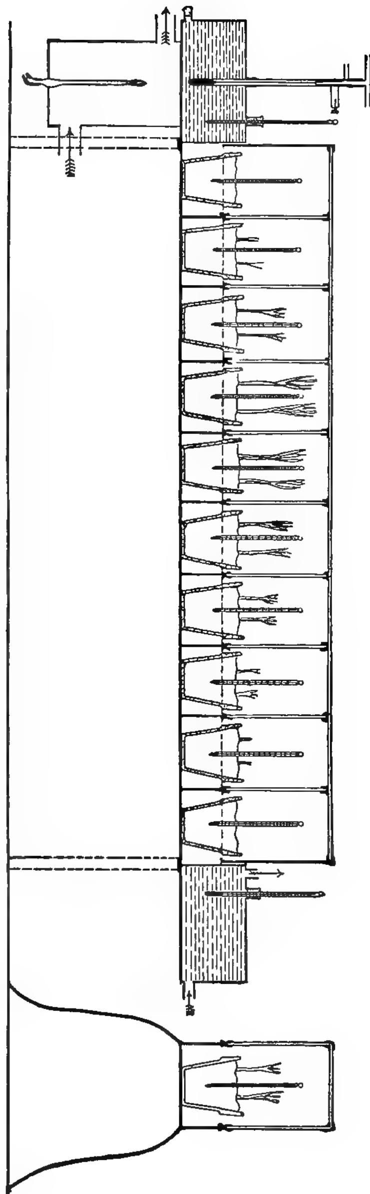


Fig. 56.—Differential thermostat; $\times \frac{1}{8}$. Explanation in text.

Growth-temperature Quantities. The great interest, both theoretical and practical, of the relations of temperature to growth has led to the making of many studies thereon, which may be traced through the pages of Pfeffer and of Jost. Taking all of these that are available for the plants commonly used in experimental work, I find that the minimum for growth ranges all the way from 0° to near 15°, with a mean at about 5°; hence the conventional minimum would be expressed as 0°–5°–15°. Similarly the optimum ranges from below 25° to about 35°, with a mean at about 30°, whence the conventional optimum of 25°–30°–35°. The maximum ranges from below 30° to somewhat over 45°, with a mean at about 40°, whence the conventional maximum is 30°–40°–45°. The conventional expression for the three cardinal points of plants commonly used in the experimental laboratory would be 5°–30°–40°.

So much for temperature. We turn next to consider the second in prominence of the variable factors to which living plants are exposed, Light, and proceed to ascertain:

What effect is produced upon growth by light?

Experiment. Prepare 3 two-inch pots of Oats, 3 of String Beans, and 3 of *Tropæolum*, or Morning-glory, as for the preceding experiment. Place them in 3 groups, each of the 3 kinds, side by side on a movable tray, and cover each group by a closed bell jar. Cover one bell jar by an opaque hood, one by a cloth hood giving about half light, and leave one uncovered; place them all in strong diffused light. Give them daily aeration and such water as they need, uncovering them only in a darkened place; and, after the seedlings are well grown, compare them as to average height, thickness, color, wealth of leaf, and other observable features.

The student should now extend his knowledge of this subject by observation of cases of light-and-dark effects occurring naturally, and by study of the books, and should make himself acquainted with present knowledge of the important subject of *the conditions of growth in darkness, with phenomena of etiolation,* and also the various kinds of effects produced by light upon growth. In this connection he should make acquaintance with MacDougal's very important work, "The Influence of Light and Darkness upon Growth and Development" (Memoirs of the New York Botanical Garden, **2**, 1903).

It will now occur to the student that light is so composite a source of energy that it will be necessary to determine to which of its constituents the resultant effects are due. The proper experimental study of the subject is of considerable practical difficulty, but if the student can undertake it, he will be aided by the following:

Suggested Experiments. Prepare 5 small pots of Oats as for the preceding experiment, and place them under, respectively, white, red, green, blue, and black pure-color screens, making all other conditions as similar as possible. Give them favorable conditions for growth, and, when well grown, compare them in all observable features of structure.

PURE-COLOR SCREENS. These have already been discussed earlier (page 112), and the statements there made as to gelatin or glass screens apply with even greater force to the present experiment. The liquid screen, first spectroscopically tested, may be applied most conveniently by use of the double bell jars of SACHS (figured in DETMER, 28), but a much less expensive substitute is afforded by cylindrical jars or bottles (figured by SACHS, "Lectures," 304, or MACDOUGAL, 234) which for the present purpose would have plain cork stoppers. With any of these arrangements ventilation should be provided either by aspirator tubes through the stoppers, or by daily renewal of the air, this being done in a dark place. The constant aspirator-ventilation is particularly advantageous because it will tend to offset the somewhat different temperatures which tend to prevail under the different colors. Or the colors can be placed in flat-sided (Soyka) flasks which are laid over flower-pots, in the bottoms of which the seeds are germinating, as described in the first edition of this book; but the method gives an insufficient amount of light to the plants, only partially compensated by the use of a mirror to reflect light directly down upon them.

We come next to the third in importance of the variable external conditions, moisture. Here it is necessary, obviously, to distinguish between moisture of the air and that of the soil, and we consider, first:

What effect is produced upon growth by atmospheric humidity?

This may be determined very simply by keeping the shoots of similar plants enclosed in chambers held at different degrees of humidity, other conditions being the same in all.

EXPERIMENT. Select 3 single-stemmed plants (*e.g.*, String Beans, Corn) in small pots, and isolate the soil either by inserting the shoots into 3 chambers with the pots outside (*e.g.*, by aid of supported bell jars, page 187), or else by enclosing the pots in shells and rubber, as for transpiration studies (page 173). Allow the air in one jar to become saturated by the transpiration, in another keep it dry by use of calcium chloride, and in the third keep it on alternate days dry and saturated; give all the plants similar and good conditions for growth. After they have grown for one week, compare the results.

The effects of varying amounts of soil moisture upon growth are perhaps too well known to require demonstration, but the student may very easily and strikingly convince himself thereon by arranging a dozen pots of Oats or other similar plants, and daily supplying to them different, but to each the same, amounts of water. In this connection, however, the student should acquaint himself from his books with our present knowledge of *the mechanical relations of water to growth, especially the rôle of osmotic pressure in forcing the enlargement of cells.*

GERMINATION OF SEEDS FOR EXPERIMENTAL PURPOSES. This is a phase of growth having a very practical experimental application. Where it is necessary to obtain roots and their hairs in a condition as perfect as possible, the best arrangement is the saucer germinator, shown by figure 57. It consists

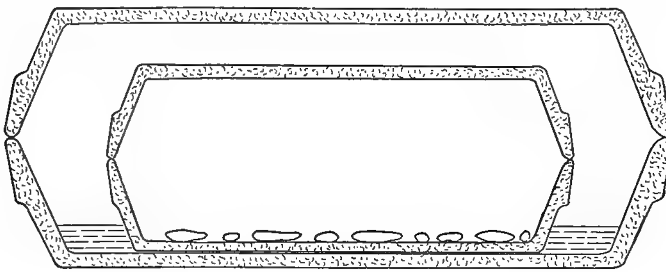


FIG. 57.—SAUCER GERMINATOR; $\times \frac{1}{2}$.
Made of four porous saucers, as explained in text.

of a cleaned, soaked, and preferably sterilized, flower-pot porous saucer holding the seeds (which are previously soaked), and covered by another of the same sort. This is placed inside a larger covered saucer which is supplied daily with water to a depth somewhat greater than the thickness of the bottom of the seed saucer. Thus the seeds are supplied with a sufficiency of water which is filtered by the saucer. The air should be renewed daily by lifting the covers for a moment, and blowing out the old air. This forms for most purposes an ideal germinator, fully as good as a Zurich or other specially constructed germinator. It has, however, for some purposes the drawback that the roots do not grow straight, nor is it useful where a considerable growth of stem also is needed. For this, and for growing seedlings generally, the very best arrangement is a flower-pot or box filled with sphagnum moss, moderately packed; this material forms the best-known medium for the purpose, as it combines almost ideal aeration and moisture, and has little tendency to develop mould. Even the little moulding it does develop can be prevented by occasional sterilization with steam, and it is advantageous to have in the laboratory a jar of sterilized moss, with another jar for that which has been used. The moss is so efficient that it is worth while to take some trouble to obtain it; it is sold by all dealers in gardeners' supplies, and may be used repeatedly. Next in value is sawdust, of which that made from pine, and hence free from tannin, is said to be best. In general the best depth to plant seeds is about three times their own (least) diameter, and they grow a little faster if the radicle end is pointed downward. Where a very straight root without any contact with moss is desired, it can be obtained by use of a moist-chamber described later under Geotropism, but here one must be always on the lookout for SACHS' "curvature," and, as far as possible, should select seeds (*e.g.*, Corn, Horse Bean, Barley, Oats) which do not raise the cotyledons above the ground.

Where it is desirable to hasten germination of seeds, it is customary first to soak them. The value, and optimum period, for this soaking for common seeds has been studied somewhat thoroughly in my laboratory by MISS ECKERSON. She has found that for most seeds a preliminary soaking does materially hasten germination, especially where these are large and are used in the saucer germinator, which of course communicates water to the seeds less readily than does moss or earth. Soaking, obviously, has no value except to hasten access of water to all parts of the seeds, and the sooner the seed is given air after this is accomplished, the better, since longer immersion actually lengthens the period of germination, and, if prolonged, causes irregularities in the seedlings. The optimum period of soaking varies from nothing in very small seeds, like mustard, up to twenty-four hours for large seeds like Beans, some of the more important of the intermediate times being, in hours, Radish 1, Barley 3, Buckwheat 3–5, Castor Bean 5, Indian Corn 7, Wheat 16. Some of the common seeds (*e.g.*, Oats, Buckwheat, Sunflower, Tomato) will germinate under water, but most others will not. Some seeds, *e.g.*, Castor Bean, Indian Corn, Wheat, String Bean, White Lupine, and Horse Bean, germinate better if the water is changed two or three times while they are being soaked. All of these times are for ordinary room temperature, and without doubt they would be shortened if lukewarm water were used. In general, with grains especially, the root end of the seed should be allowed air, no matter if the remainder is immersed; but after the roots have developed they will grow freely, as a rule, even under water.

An incidental advantage of soaking is that soaked seeds develop fewer moulds than unsoaked, of course, because the soaking removes the spores. Hence when seeds are not soaked, it is well to wash them before use. Methods of growing seedlings without moulds, in aerated test-tubes, have been described by METCALF and HEDGCOCK in Journal of Applied Microscopy, 6, 1903, 2493. Further, immersion for 10 minutes in water kept between 56° and 57° is said to kill smut (and doubtless other Fungi) of Oats.

A number of simple germination methods, suitable for elementary demonstration purposes, are described by LOOMIS in the Nature Study Review, 3, 1907, 200. Compare also E. F. BIGELOW's notes in the same journal.

Any consideration of the effects of heat and light suggests the third leading form of energy, *viz.*, electricity, including magnetism. Since plants are not exposed to these forces in Nature except in the rare and violent form of lightning, or in the necessarily weak form where light plays upon leaves, any effect they have upon growth can only be of an accidental or coincidental character. The practical difficulties in the experimentation, however, are very considerable, and the student may most profitably work up the subject from the literature, which includes a valuable reference and summary in the Botanical Gazette, **45**, 1908, 286.

Of the other variable external conditions, the most prominent is barometric pressure, which has two forms of fluctuation, *viz.*, daily in correlation with weather changes, and altitudinal with elevation above sea-level. Here again the experimental difficulties will send the student to the literature. Variable chemical agents, such as special gases in the air, etc., have already been considered under their effects upon protoplasm. Variable food supply is perhaps of too obvious effect to need experimental study, but its influence can be shown very readily by the simple and well-known method of comparing the growth of three differently treated sets of seedlings developing from seeds having the food stored in the cotyledons (Beans, Peas, etc.); both cotyledons are removed from the plants of one set, one is removed from each of those of another set, and none are removed from the third. To some extent the comparative growth of very large and very small seeds selected from the same stock proves the same fact.

The consideration of food supply for Growth involves the problem of energy supply, a matter which has already been studied, for growing tissues, under Respiration. The student should now review that work from the present point of view, and extend it to an accurate knowledge of *the relations of Respiration to Growth.* The experimental study, or demonstration, of the dependence of Growth upon Respiration is easy, needing simply some such arrangement as that already described (page 126), by which two sets of similar growing tissues are, respectively, supplied with, and deprived of, air containing oxygen. Here also the student should inform himself upon the other *physical and chemical phenomena accompanying Growth.* One physical phenomenon of importance is the power of growing parts to exert much pressure, developed through osmosis, a subject on which some simple experimentation is practicable.

Suggested Experiments. Construct from small bored-and-split corks, held by rubber bands, a series of small pressure-jackets which will just fit on the tips of strong roots growing in sphagnum moss; and ascertain what tension, in grams, can just be overcome by the swelling root.

Some ingenious experiments to the same general end, but upon a different plan, are described by Osterhout, 73.

A somewhat important phase of the relations of osmotic pressure to Growth concerns the relative tensions of the tissues in young shoots, a subject which will be considered later, but which may receive practical illustration as follows:

Suggested Experiments. Select a young, stout, firm-growing internode some 100 mm. long, and measure its exact length; then, with a sharp knife, quickly strip off a band of epidermis of the full length, and immediately measure it; then strip off the entire woody tissues in four or five cuts, and measure a piece of this; finally measure also the core of pith.

Also select the stoutest young internode available, and peel off a ring of epidermis. Immediately replace it and note the alteration of size. *Or* use a young branch and remove a ring of cortex down to the cambium. Also, select a firm herbaceous internode; quickly split it lengthwise through the middle, and note the curvatures.

The foregoing study will show the remarkable relative tensions which bring young growing plants into a condition of unstable internal strain. This suggests that the direction of longitudinal growth might be altered by very slight causes, and thus raises a question for practical study.

Do slender growing parts under ordinary conditions grow straight in the line of their axes, or do they move about (circumnutate?

This may be determined by arranging for frequent exact observations of the positions of such parts. But as any movements must be slight, it is necessary to ensure their magnification.

Experiment. Select a seedling or other shoot in rapid growth. Prepare, by drawing out glass tubing in the Bunsen flame to capillary fineness, a very slender straight glass filament; at one end of a piece 2 cm. long put a minute drop of black sealing-wax, and a little way over the other end slip a paper circle 5 mm. in diameter, with a very small black spot in the center, through which the filament passes; attach this end by thick shellac dissolved in alcohol to the tip of the stem of seedling or shoot, and hold it until set. Then attach a similar filament to the tip of a young leaf standing horizontally. Then place the plant, uniformly illuminated, with the tips of each of the filaments just 25 cm. beneath a sheet of glass supported at right angles to its axis. Holding the eye a convenient distance (also about 25 cm.) away from the glass, sight along the filament until the black spots coincide, then make on the glass, in the line of sight, a small dot, using for the purpose a needle with a wax tip dipped in India ink. Then thereafter, at as frequent intervals as practicable for three or four days, make similar records of the position of the filament, numbering each mark. Finally take off on tracing-linen a copy of the dots, join them by fine straight lines, indicate the direction of movement by arrow points, and state each time interval in minutes.

Precautions. In attaching the filament the smallest efficient quantity of shellac should be used. Further the plant must be screened from one-sided light. This is accomplished automatically in a well-lighted greenhouse, especially if the south side is shaded, but in a room it is necessary to surround the plant up to near the glass by a paper cylinder, so that all light shall fall from above, a small opening being left in the side for the observation of the leaf filament. A good arrangement, where only the movement of the main stem is desired, is afforded by placing the part under observation in the bottom of a box, or even a large flower-pot, on the top of which rests the glass plate. While it is customary to join the dots by straight lines, there is some advantage in using sweeping curves through the points, though one system is about as true, or untrue, to Nature as another. While a support for the glass plates may readily be adapted from the ordinary laboratory supports and clamps, it is much more satisfactory to use a tripod made for the purpose, after the plan shown by the accompanying figure (Fig. 58), the whole being placed upon a low table or other support so that both plates may readily be used. Instead of the circle of paper above described, one may use a triangle of paper with the dot on one angle, or even may place the dotted paper on a stick set in the pot,—any arrangement which permits two dots to be seen and brought into line sufficing for the purpose. But upon the whole the arrangement above described is the best, since it permits the record mark on the glass to be made just where the filament,

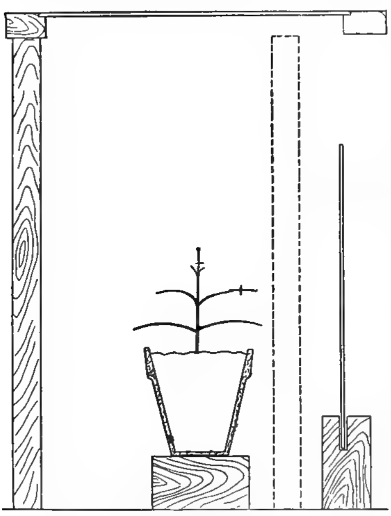


Fig. 58.—Arrangement for the study of circumnutation; $\times \frac{1}{8}$.

The upright glass on the right, for convenience of the drawing, is shown too near the plant. Both glasses are (preferably) circular.

and therefore the stem (or leaf), points. The adoption of 25 cm. (10 inches) as a standard distance from filament to glass has the advantage of permitting different records to be directly compared.

Other Methods of Studying Circumnutation. The above method was introduced by Darwin (described in his book, "The Power of Movement in Plants"), and has not been improved since his time. It has obvious drawbacks, one of the most serious being the error introduced into the record as the filament points out more and more obliquely to the plate. This error can be largely overcome by use of a curved glass with the filament lying in the radius. Such glasses can be obtained, and when placed, for example, upon the tops of flower-pots which have seedlings growing near their bottoms, yield very satisfactory records; but they have the drawback that these records cannot be transferred to a flat surface for preservation or publication. Another method of preventing this error is that of Wiesner (Bewegungsvermögen der Pflanzen, 1881), who used a blackened tube, with cross wires, resting upon a glass plate; this is slipped about until the filament or tip is covered by the cross of the wires, when the record mark is made upon a second glass plate placed beneath the first. A modification of his method, requiring only a single plate, has been described by Stone in the Botanical Gazette, 22, 1896, 262. A quite different method of recording circumnutation was that of Dewèvre and Bordage (Revue générale de Botanique, 4, 1892, 65), who photographed at intervals the position of the whitened tip of the filament, but the method is of very moderate value. Still another distinct method is that of Fritzsche, described in a paper which is the only important recent work upon the subject (Ueber die Beeinflussung der Circumnutation durch verschiedene Faktoren, Leipzig, Oswald Schmidt, 1899). He used a microscope arranged to be moved by screws above the parts studied, and followed the movements directly upon an ocular micrometer ruled in squares, a record being made upon the corresponding squares of a ruled paper. It is also possible to study the circumnutation of roots. This can be done by growing them upon inclined smoked-glass plates, as Darwin did ("Power of Movement in Plants"). But it can also be accomplished by more direct methods as follows. Prepare a cylindrical glass moist-chamber like that shown in figure 59, and arrange a strong young root to grow in its axis. Down the outside of the chamber, 90° of the circumference apart, rule two fine black lines; then, facing these lines and 25 cm. from the root, set up two glass plates, so that it will be possible to sight through them and record the position of the roots as brought into coincidence with the black line beyond. Thus an accurate record of the nutation is obtainable. Of course the chamber should be kept darkened except while the actual record is being made.

Materials. These, for the plants accessible to the American teacher, have been studied fully in my laboratory by Miss Hope Sherman, who has used both Darwin's original method and Fritzsche's microscope method. She has confirmed the well-known fact that seedlings have a more active movement than older plants, and that in general the more slender a part is, the greater is its movement. Of plants readily available for the study, the Horse Bean shows the most active movement, though for a combination of rapidity, amplitude, and frequency of change of direction, the Radish is best of all. Then follow in order Fuchsia, Grape Hyacinth, Sunflower, Ficus, and Oats.

Demonstration Methods. Circumnutation, it is true, is a phenomenon of no great physiological importance, but it is worth some emphasis in an experimental course because it is always a matter of general interest, and, moreover, it involves some very exact manipulation which affords valuable training even to comparatively young students. I find the method above described, using the apparatus of figure 58, very satisfactory.

The student should now, by aid of the literature, extend his study of this subject to an understanding of our knowledge of *the nature and meaning of Circumnutation* (which would better be called Nutation) *in relation to Growth, together with other autonomic growth movements.* Especially he should make some study of Darwin's book, "The Power of Movement in Plants."

The consideration of Nutation leads to the inquiry whether other movements exist, likewise dependent upon internal, rather than external, factors. Several of these do occur, and (aside from the anomalous spontaneous movements of the leaflets of *Desmodium gyrans*) are mostly of much importance to the assumption of its form by the plant. These include *epinasty, hyponasty, and, under exceptional conditions, rectipetality (or autotropism).* Of another kind, though also belonging here, are *polarity, some phases of regeneration, and Sachs' curvature.* All of these are susceptible of ready observational or experimental study, and the student may follow them, if he will, by aid of the excellent directions given by Detmer, to which I am unable to add anything from my own experience. Many other growth movements there are, but these depend upon external stimuli, and hence their study belongs under Irritability later.

There still remain some special topics of more or less importance which, however, the student may most profitably work up through the literature. They include *resting periods (and the relation thereto of enzymes), rhythms, and periodicity* (especially striking in the vegetation of temperate climates), contractions during the growth of some roots, and a relation between transpiration and growth whereby one may to some extent be used as a measure of the other (on which consult LIVINGSTON, Botanical Gazette, **40**, 1905, 178, and Science, **22**, 1905, 146). There is also the whole important subject of the toxicity of various substances to growing roots, which has been earlier touched upon (page 162), and on which there are some very important contributions in recent volumes of the Botanical Gazette. Finally there is the subject of growth from the minute or molecular point of view, involving the nature of the growth of solid structures such as cell walls and starch grains, and the modes by which they are modified after their formation, inclusive of sliding growth.

(*b*) *Differentiation.*

Thus far under Growth we have been considering increase of size only; now we turn to the other important phase of the subject, differentiation of new parts. In reality this has itself two phases: first, the formation of new cells, which usually precedes increase in size, and, second, the formation of permanent tissues of special function after the increase of size has been accomplished. Important though this subject is, it yet admits of little practicable experiment, and the student must work it up through the literature, in which he should give especial attention to the following matters:

(*a*) The physiological relation of the cell to the organism, as to which is the physiological unit (compare WHITMAN on "The Inadequacy of the Cell Theory," in the Journal of Morphology, **8**, 639).

(*b*) The distinction in cell formation between differentiation and auxesis. (This may very readily be studied observationally and experimentally, to which end the embryos and seedlings of young succulents, especially *Cactaceæ*, are admirable.)

(*c*) The significance and determinants of cell size in relation to body size.

(*d*) The relations of the positions, directions, and numbers of cell divisions to adult form.

(*e*) Seat and nature of the control over these divisions, involving the mechanism of heredity.

(*f*) Mode and place of origin of new parts, leaf, stem, root, bud.

(*g*) The hereditary ground form, and deviations therefrom in torsions, fasciations, etc., and extent to which it is modifiable by experiment. (On this subject GOEBEL'S new "Einleitung in die Experimentelle Morphologie," Leipzig, Teubner, 1908, will no doubt rank as the standard work.)

LITERATURE OF GROWTH. The subject is thoroughly summarized by PFEFFER and by JOST, and there are no papers of more recent date, aside from those cited, so far as I have found.

10. REPRODUCTION.

Very closely bound up with Growth, in the lower plants coincident with it, and in the higher simply a special phase of it, is that other form of Increase called Reproduction. Its physiological phenomena are vastly important, but they do not admit of profitable experimental study in such a course as this. Some of them are bound up with cytology, others are so recondite as to be open only to very refined investigation, while others belong rather to that special phase of physiology called ecology. Accordingly the student may most profitably work up the subject through the accessible literature, concentrating his attention upon the following matters:

(*a*) The fundamental (or philosophical) relation of Reproduction to Growth, involving the physiological relations of parent to offspring.
(*b*) The physical basis of reproduction in cell, nucleus, and chromosome, both as to divisions and fusions.
(*c*) The relation of the ontogeny to the phylogeny of an individual.
(*d*) The physiological characteristics, with the probable origin and phylogeny of asexual and of sexual reproduction, this involving the advantages of fertilization and the meaning of sex.
(*e*) The mechanical and physiological evolution of fertilization mechanisms, and the limits of sexuality in the higher plants.
(*f*) The probable advantages of cross, contrasted with close, fertilization, and the mechanisms it has developed.
(*g*) The nature and significance of variation, rejuvenation, and senescence.
(*h*) The significance of the special features, parthenogenesis, xenia, double fertilization, independence of grafted parts, alternation of generations.

SECTION 3. THE PROCESSES OF ADJUSTMENT.

Having considered the ways by which Plants maintain their individual lives, and also by which they make increase, it remains to study the third of the primal activities,—their adjustment to their surroundings. Of this there are two phases which are undoubtedly in some way connected, though by a bond at present unknown, *viz.*, *first*, adjustment of the individual through definite responses to the forces of the environment acting as stimuli, that is, *Irritable Response*, and, *second*, adjustment of the race to its environment, fixed in heredity, that is, *Adaptation.*

11. IRRITABLE RESPONSE.

Already in this course the student has more than once come close to contact with cases in which the plant responds adaptively to external conditions, and, indeed, the subject was definitely, though briefly, discussed when considering the influence of external forces upon Protoplasm. We must now investigate this subject more exactly, especially with reference to the nature of the responses. The external forces have already been enumerated (page 65), and it will be well to begin with the one in which it happens that the nature of the relation of stimulus and response is most clearly exhibited; this is Gravitation, the response to which introduces us to Geotropism.

(*a*) *Geotropism.*

The most familiar case of geotropism is doubtless the assumption of the invariable up-and-down position of stems and roots respectively as they grow from germinating seeds, regardless of the position of the seeds, and our study may well begin by exact observation of this phenomenon.

What are the observable geotropic phenomena exhibited by the young parts of developing plants?

EXPERIMENT. In a suitable moist-chamber, accessible to observation, fix a half dozen well-soaked large seeds, preferably of a kind from which the cotyledons are not raised (Horse Bean, Corn), in positions as different as possible. Give favorable conditions for growth, and observe the positions taken.

As soon as the roots are 2–3 cm. long, swing the plants in their respective planes through 45° from the vertical, and later, after they have grown somewhat longer, swing them to 90°, and observe results.

MOIST-CHAMBERS. These are of very diverse forms according to their particular uses, but all in common should provide a constant water-supply with a saturated atmosphere, darkness with ready accessibility to observation, and good aeration. A simple form is a germination box (originally described by SACHS), *viz.*, a sphagnum- or sawdust-filled box having a sloping glass side; if the seeds are planted against the glass, well above the middle, the developing roots will show clearly against the glass. The box may well be made of wire netting of shallow form, with the glass as a removable cover, the whole being stood against a support with the glass sloping. *Or* a large funnel in upright position is a good substitute. Another very excellent form, especially useful for demonstration purposes, is made from two sheets of glass holding between them a sheet of soft dark-colored felt paper (herbarium dryers) saturated with water (described by PEPOON in School Science, 2, 1902, 179). Other forms of the same type (often called Root Cages) are described by BARNES in his "Plant Life" (Holt & Co., 1898), 200, and by LLOYD in the Plant World, 8, 1905, 262. The seeds are placed in any desired positions between paper and glass, and, with added bits of paper at the corners to prevent too great compression of the seeds, the glasses and paper are clamped together by wooden clothes-pins. A common form of moist-chamber is a glass box or jar lined in whole or in part by filter paper kept saturated, and even a glass-stoppered bottle, with water in the bottom, is sufficient for many purposes. A good form is described by REED in Journal of Applied Microscopy, 4, 1901, p. 1499, and 5, 1902, p. 1890. SACHS has also described a very complete moist-chamber for geotropic studies (Flora, 80, 1895, 293). In most respects an ideal moist-chamber is formed by a clean porous flower-pot in a saucer of water, either inverted with the hole stoppered, or in ordinary position, with a saucer of water also as cover; the whole arrangement thus has evaporating walls, and incidentally is markedly cooled in hot times by the external evaporation. It may be made even better by building a circular dam of modelling-wax on the inverted bottom, thus forming a saucer in which water may be kept standing, to the more ready saturation of the pot, especially if this be large. Aeration, for most purposes, is amply provided by a daily blowing out, but it would be very easy to arrange an aspirator to draw in saturated air. The one drawback to this chamber is that its contents are not directly accessible to observation, a difficulty which may be partially compensated by the use of openings drilled in the sides and kept stoppered when not needed for observation, or covered by glass windows cemented into the sides. But where frequent observation of the entire object is desirable, as in the present experiment, a better chamber is that shown by the accompanying figure (Fig. 59), made from a battery-jar with water on the bottom, and a water-filled porous saucer for a top, the seeds being supported on corks and a tumbler, and kept wet by cotton wicking, as shown by the figure. The plants may be swung through 45° and 90° by loosening the pins and resetting them in the new position. The whole arrangement is enclosed by a suitable removable hood, and aeration is provided by a daily blowing out. In this chamber the seeds germinate well-nigh ideally and show well the positions taken by the new parts. In such chambers the seeds may be supported simply by pins holding them to a cork, with interposed cotton wicking, as in figure 59, or may be inserted between folds of wet filter-paper held by rubber bands against a bar of wood, as recommended by REED, or in a cage made of wire netting (dipped in melted hard paraffin to make the metal innocuous), filled with moist sphagnum, as I have used with success.

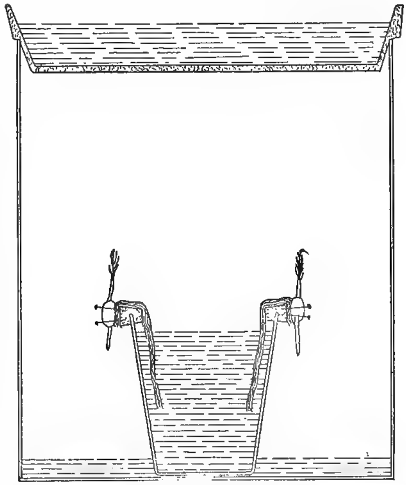


FIG. 59.—GLASS MOIST-CHAMBER FOR STUDY OF GEOTROPISM; $\times \frac{1}{2}$.

The dark cover is omitted. Further explanation in text.

DEMONSTRATION METHODS. The use of the glass-and-felt-paper chamber above mentioned, using Corn, Oats, or Barley seeds, gives the best, and a well-nigh ideal, simple demonstration for elementary purposes, especially as it so easily allows of change of position of the roots and stems. Of course much can be shown as to stems by simply laying potted plants upon their sides, when a very marked response occurs in a few hours. Plants completely inverted give striking results.

The results of the foregoing experiment leave no doubt that growing parts assume an up-and-down position quite regardless of the positions of the parts from which they start. The sole determinant of up and down, under the conditions of the experiment, is gravitation, which, therefore, would seem to be the guiding influence. This raises an interesting correlative problem as follows:

What effect is produced upon young growing parts if gravitation is not allowed to influence them?

Obviously it is not possible to remove any object from the influence of gravitation, but it is possible to use a substitute method, *viz.*, one by which gravitation is felt equally from all sides, as occurs when the object is continuously rotated in a vertical plane.

EXPERIMENT. To the rim of the disc of a klinostat revolving in a moist-chamber, fasten 5 or 6 corks after the manner shown in figure 60. To each of these pin a soaked seed of the kind used in the foregoing experiment, placing between seed and cork a short piece of cotton wicking arranged to dip at each revolution into a dish of water, thus keeping the seeds wet. Observe the growing parts and ascertain the determinants of the positions taken.

A large flower-pot, standing in a saucer of water and covered by another water-containing saucer, makes an ideal moist-chamber for this purpose. A hole for the klinostat rod may easily be drilled by a file turned in a carpenter's brace. The water may be supplied by stopping the hole in the bottom of the pot and filling it to the desired depth.

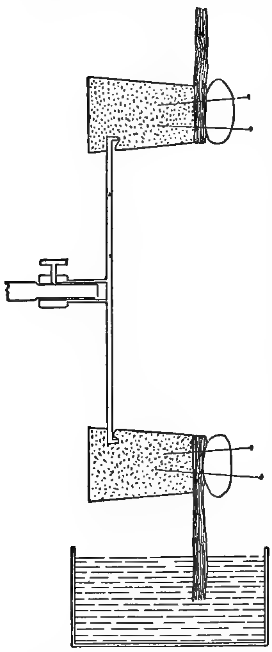


FIG. 60.—ARRANGEMENT FOR REVOLVING SEEDLINGS BY THE KLINOSTAT; $\times\frac{1}{2}$. Particulars in text.

KLINOSTATS (OR CLINOSTATS). The Klinostat is an indispensable instrument for any thorough work not only upon geotropism, but also upon other phases of the irritability of plants. The requisites of a good Klinostat are: (*a*) it should carry plants of considerable weight; (*b*) should carry them either horizontally, vertically, or at any intermediate angle; (*c*) should revolve completely (preferably at a speed variable at will), not faster than once a minute or slower than once in thirty minutes (this time bringing the revolution within the reaction time of a responding structure without introducing centrifugal force); and (*d*) should revolve uniformly even with somewhat irregularly balanced load, the uniformity being important since any regularly recurring retardation permits a summation of stimuli, with their appropriate response, in that position. The original Klinostat invented by SACHS in 1872 was driven by a weight-and-pendulum clock, and carried but light loads. The standard instrument of recent years has been PFEFFER'S (figured in his "Physiology," 3, 169), which is supplied by Albrecht of Tübingen at a cost of 350 marks. It is driven by a powerful spring controlled by a fan governor, and depends upon accurate balancing of the load to ensure uniformity of revolution. Similar in principle, though very different in details of construction, is the WORTMANN Klinostat (pictured in DETMER, 455), supplied by Ungerer Bros. of Strasburg at a cost of about 200 marks.

I find it needs to be kept in the most perfect condition to work properly. A small form, with escapement regulator, suitable only for seedlings or plants of very light weight, is that of HANSEN (Flora, **84**, 1897, 353), costing 115 marks, but it has serious practical limitations. Recently a new and very different form has been described by NEWCOMBE (Botanical Gazette, **38**, 1904, 427); it is driven by a small electric or water motor, whose speed is stopped down by suitable worm-gear or pulleys. It has the advantages of simplicity in construction, moderate cost, possibility of connecting several Klinostats in series to one motor, and especially of so much power that any retarding effect due to irregular distribution of weight is wholly eliminated. Another electrically driven form of different details of construction has also been described by VAN HARREVELD (Die Unzulänglichkeit der heutigen Klinostaten für reizphysiologische Untersuchungen, Groningen, Holland, M. de Waal, 1907). This electrically driven type is likely to form the standard instrument for research in the future. Its need for a constant electric current, or constant head of water, obviously limits its usefulness for educational purposes, and in order to provide a suitable form for educational demonstration, I have designed the instrument which is supplied among my normal apparatus and is figured herewith (Figs. 61, 62, 63). Though intended primarily for educational use, for which it is ample, it is incidentally entirely suitable for some investigation purposes where light weights are concerned, or where these are carried with the axis vertical. It consists essentially of the works of a powerful eight-day clock, geared to a revolution in fifteen minutes (this being the only speed required for educational demonstration and for most investigation) and enclosed in a practically dust- and moisture-proof case, 16 cm. in diameter. It can be used in any position whatever, as shown by the accompanying figures. It has been greatly improved over the original form (described in the Botanical Gazette, **37**, 1904, 304) by the addition of a ball-bearing shaft which takes the strain from the works, while friction-rollers have been added to the spindle-rod support. The

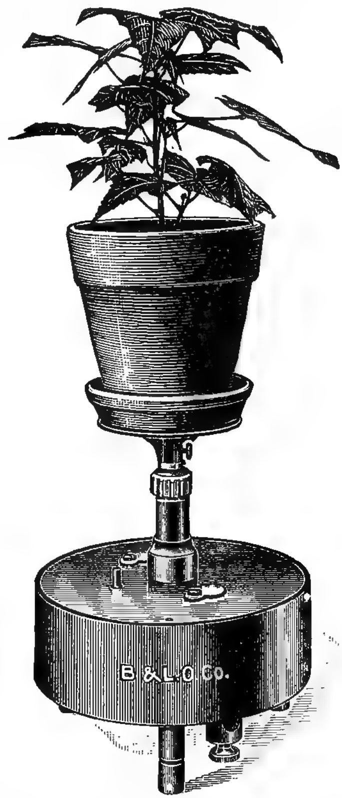


FIG. 61.—DEMONSTRATION KLINOSTAT; ×¼.

works are started and stopped by a cylindrical nut projecting from the upper surface. It is to be wound, and not too tightly, once in two days, which may be accomplished without disturbing the plant. Properly used it will carry a 4-inch or smaller pot horizontally, or a larger size vertically. As with all Klinostats, however, it revolves with the greater evenness the smaller the weight it has to carry, and consequently the smallest pots allowed by the subject under study should be used. The size with which the instrument works best is the "three-inch," this size, however, being larger than commonly supposed, since the measurements of pots are all internal.

In using the Klinostat with plant vertical (Fig. 61), it should be levelled by means of the extensible legs; a 4-inch flower-pot saucer should be stood

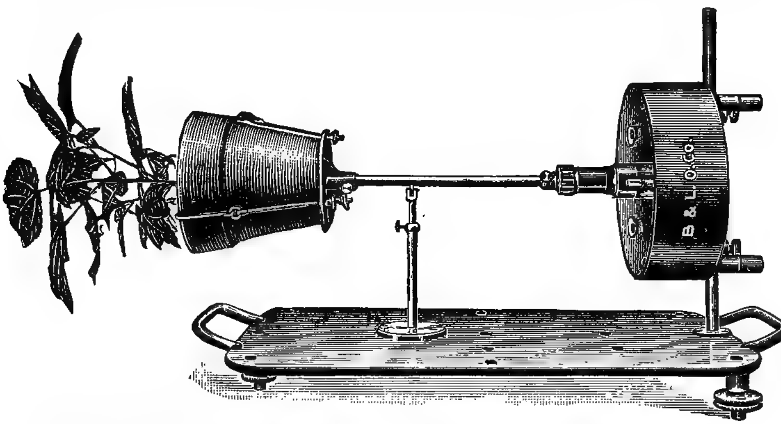


Fig. 62.—Demonstration Klinostat arranged on special support stand; $\times \frac{1}{6}$.

on the disc, into the rim of which it exactly fits, to catch the drip from the pot, and the plant should be stood exactly in its center. In using the Klinostat with plant horizontal, the general arrangement of figure 62, where it is shown supported upon a special portable clamp stand, is advantageous, though any upright support may be used if firm enough to hold the Klinostat in a truly vertical position. The weight of the plant must be centered in order that the instrument may be able to keep the revolution uniform throughout. This adjustment is effected thus: The pot is attached to the disc by means of the screw-rods, which, however, are not tightened. The plant with the spindle-arm is then placed in position, resting on the extensible spindle-rod support, as shown in the figure, but the screw attaching the spindle-arm to the clockwork is left loose. The plant is then given a twirl with the hand, and after turning once or twice comes to rest with its heaviest side down. The pot is now pushed on the disc from the heavy side and twirled again, and the process is repeated until, after a few trials, the plant comes to rest indifferently in any position. Then the screw-rods, and finally the screw connecting the spindle-arm and works, are tightened, and the experiment may begin. Care must be taken never to allow the weight of the plant to be unsupported, else the spindle-rod may be greatly bent and damaged. Further the adjustable support must be neither too high nor too low, else the spindle-rod may "bind" and refuse to turn. It is very rarely, if ever, in educational work that any position other than horizontal or vertical is needed, but if desired it may be attained as in figure 63, the adjustment being so made that one of the legs of the instrument rests firmly on the support or table. This position brings much more strain upon the works than either of the others, and consequently should be employed as rarely, and with as light weights, as possible. In using the instrument the following precautions should be observed. No water should be allowed under any circumstances to get into the works, and the instrument should be exposed to as little moisture as possible. It should also be kept constantly closed from the dust. Sudden or extreme strains on the works must be avoided. Like all clockwork it must be cleaned and oiled at intervals, the length of which depends upon the amount of use.

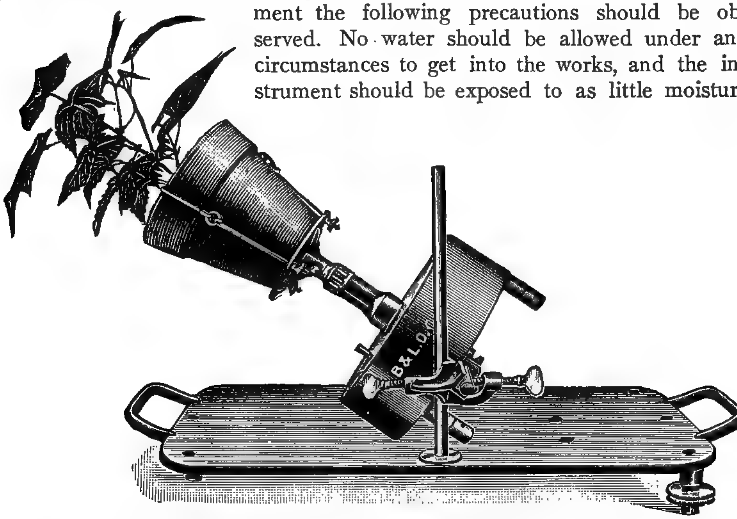


FIG. 63.—DEMONSTRATION KLINOSTAT ARRANGED FOR USE AT AN ANGLE; $\times \frac{1}{8}$.

A form superficially resembling the instrument just described is supplied by the Cambridge Botanical Supply Company, and another, of somewhat similar construction, is offered by the Stoelting Company. Yet another, driven by a spring clock, but having a new form of support (a right-angled frame carrying both clock and the revolving object), is offered by the Cambridge (England) Scientific Instrument Company. Klinostats giving an intermittent revolution, needed for some special purposes, have been described by F. DARWIN (Annals of Botany, 6, 1892, 245), by PFEFFER (Jahrbücher für wissenschaftliche Botanik, 35, 1900, 738), and by FITTING (PFEFFER, "Physiology," 3, 109).

The Klinostats so far described are either normal or precision forms, but a number of adapted types have been invented. Thus aside from a crude Klinostat centrifuge, made from clock works, described by SWEZEY in the Botanical Gazette, **16**, 1891, 147, STEVENS has described a simple form run by a water-motor (Botanical Gazette, **20**, 1895, 92); STONE gives one run by clockwork (Botanical Gazette, **22**, 1896, 259), and I have myself described a good form, constructed from the works of a powerful clock, in the Botanical Gazette, **27**, 1899, 258, an instrument which, by the way,

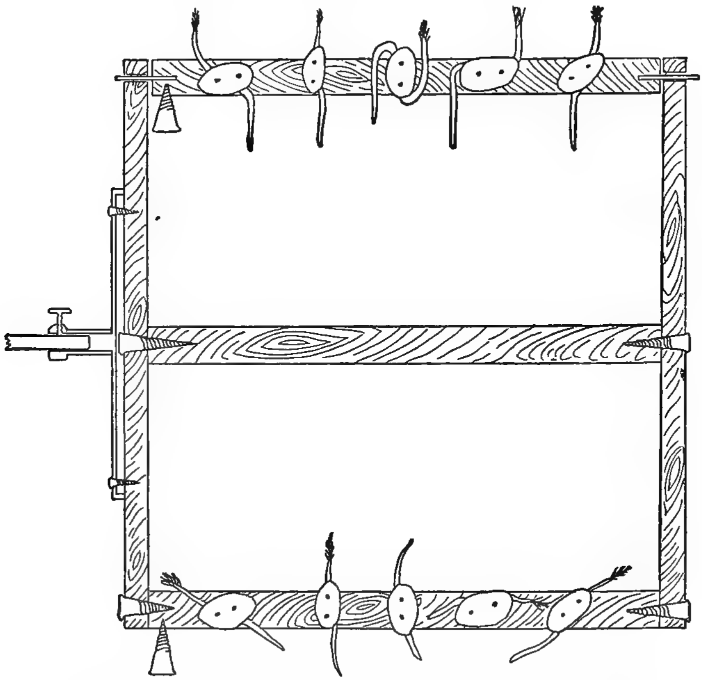


FIG. 64.—FRAME FOR DEMONSTRATION OF GEOTROPISM; ×½. Explanation in text. The lower counter-weight should be shown hanging on a wire loop which turns on the frame.

would be much improved by using a box of wire netting instead of the crystallizing dish. Still simpler arrangements approaching the make-shift character, and revolving but once an hour (which is sufficient for some of the simplest uses), have been described by OSTERHOUT, 91, by STEVENS in his "Introduction to Botany" (Heath & Co., 1902), 25, and by others.

The limitations of Klinostats, and the proper principles in their construction and use, have recently been discussed by NEWCOMBE in Science, **20**, 1904, 376, and by VAN HARREVELD in his paper cited above.

Demonstration Methods. The influence of gravitation in determining the direction of growth of young parts may most effectively be shown by growing similar seeds under conditions precisely alike except that one set is kept revolving in a vertical plane, while the other keeps an upright position. This may be accomplished simply and very effectively by the arrangement figured herewith (Fig. 64), which may be constructed from wood in the laboratory, and will later be supplied in efficient form among my normal apparatus. It consists of a wooden frame revolving in a darkened moist-chamber on the supported end of a Klinostat rod and dipping its bars under water at each turn. One of the bars is fixed to the frame, but the other is freely movable on pivots, and, moreover, has a weight which keeps it always in one position relative to gravitation. If, now, sets of seeds are pinned in corresponding positions to the two bars (half on one side and half on the other for better balancing), and the Klinostat is set in motion, the two sets are under precisely similar conditions, even to being watered alike, except that one set is always in the same position as to gravitation, while the other is constantly changing. In fact this method leaves little to be desired in its effectiveness of demonstration of this subject. Methods of demonstrating geotropic and other movements to audiences by projection are described by Pfeffer in a paper earlier cited (page 23).

The foregoing experiments are concerned with the determinants of the positions of the main root and main stem, leaving it still to be ascertained:

Is gravitation concerned in determining the positions of side roots and stems?

Experiment. In a moist-chamber of the germination-box, or glass-and-paper, type, germinate seeds of a kind in which the young seedlings early develop side roots (*e.g.*, Bush Bean, Radish), and, as soon as the side roots are from 1 to 2 cm. long, swing the glass plate in the same plane through 45°, and observe the results. Later restore it to the old position. (It will also be of interest to revolve the plants on the Klinostat in the plane of the glass.)

Experiment. Select a potted plant having slender growing side branches, and, tying the main stem to a rod thrust into the earth, tip pot and rod to 45° from the vertical, and observe the position of further growth of the side branches.

These studies will suggest a further inquiry as to whether other parts of the plant, leaves, flowers, tendrils, etc., are geotropic, matters which the student may in part settle for himself by aid of the following:

Suggested Experiments. Select a plant with slender-growing petioles (*e.g.*, Garden Nasturtium); tie its main stem to a glass rod; place pot and rod horizontal in the dark, and observe positions taken by the leaf blades. Plants with a pulvinus to the leaf (*e.g.*, String Bean), and stemless plants like Oxalis, give marked responses to a corresponding treatment. Select a potted plant which has a cluster of irregular flowers (*e.g.*, Larkspur, Garden Nasturtium); bend over the tip of the cluster until it is inverted and tie it in that position; keep in darkness, and observe positions of the opening flowers. *Or* shoots, preferably cut under water and kept in water in a dark room with tip inverted, give good results. The flowers of Narcissus, and the buds, in relation to the open flowers, of Poppies, are important objects for this study.

Select a young potted twining plant; observe the method of twining; lay it down horizontally and note the twining phenomena. Also, if practicable, revolve such a plant upon the klinostat, and note and interpret the effect upon the twining.

The student should now work out, from all his sources of information, a good exposition of *the occurrence of geotropism in plants, including geotaxis and prominent cases of positive, negative, lateral, transverse, and other special manifestations of geotropism, with their significance in the economy of the organism.*

In the course of his observations the student will have noticed some cases of rather rapid responses, and it is important to know how rapid the response may be. This can readily be determined thus:

SUGGESTED EXPERIMENT. Arrange 2 or 3 strong-growing seeds as for the first geotropism experiment, and, when the roots are 1 or 2 cm. long, turn them into a horizontal position, preferably projected against a background ruled in small squares. Keep the chamber at a temperature of 22° to 25°, and observe the roots frequently, noting the time needed to show the first sign of bending, and also that required for complete turning. (A special chamber for this purpose is that of SACHS, mentioned under Moist-chambers, page 220. The experiment works well if the seeds are held in a wire-and-sphagnum cage placed inside a flower-pot moist-chamber.)

Place a slender-stemmed actively growing plant on its side, and note the time needed for a first trace of bending and for complete bending.

The student should now follow through the literature our knowledge of this subject, including with it the related matters of after effects, and especially *the correlations existing between the geotropism of main and of side roots or stems.* It is quite possible to study these correlations experimentally by using SACHS' simple device of severing the main root some distance behind the tip, and observing the effect upon the neighboring lateral roots.

Observation of the rate of response raises the query whether the bending is a passive and easily resisted process, or one which proceeds with some force, and we ask:

Against what resistance can geotropic response take place?

EXPERIMENT. Prepare a flotation dynamometer as shown by the accompanying figure 65, the essential part of which is a vial float resting upon mercury and sliding in a cylindrical vessel, the float being guided in the vessel with a minimum of friction by pins attached by sealing-wax to the float. Firmly support a seed above the vial, with the root, germinated to about 1 cm. length, pressing into a sealed glass tube set in the cork; give water-supply, place in a flower-pot moist-chamber, and observe how deeply the root can push the float before beginning to bend, a point which can be registered by placing on the mercury a little moistened cork dust, which will leave traces at the highest point reached. Then remove the seed and add weights to find the pressure in grams needed to depress the float to the same depth.

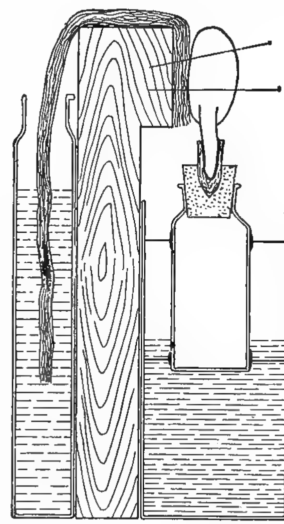


FIG. 65.—FLOTATION DYNAMOMETER, FOR MEASUREMENT OF THE GEOTROPIC GROWTH POWER OF ROOTS; × ½.

Explanation in text. The size of the float must be adjusted to the power of the root.

OTHER METHODS OF STUDY. A method of measuring the force of geotropic response was devised by SACHS, who made the growing roots press against tiny scale-pans connected over a pulley with small weights. A modification of this, with a special wire cage for the root tip, is described by STONE in the Botanical Gazette, 22, 1896, 293. For demonstration purposes SACHS' familiar method of allowing a horizontally extended root to turn into mercury (covered with a film of water) is rather effective, the upward bend of the root behind the tip, as well as the depth of penetration, giving some qualitative idea of the resistance. This experiment may very conveniently be applied by aid of a hollowed wooden block used as shown by the accompanying figure (Fig. 66); or it may simply be hollowed by two auger holes of differing size and depth. A dynamometer for such measurements is described and figured by DETMER, 447, 532, by MACDOUGAL, 25, by STONE, Botanical Gazette, 22, 1896, 251, while a very exact instrument adaptable to this purpose has been described by PFEFFER (figured in DARWIN and ACTON, 132).

The power exerted in geotropic response, together with the very obvious lengthening which accompanies the bending of roots and stems, suggests that the process must be ultimately connected with Growth, and the problem arises:

Are ordinary geotropic responses dependent upon growth?

This may be determined in either of two ways: *first*, by arranging all conditions for a geotropic response, but withholding some essential condition of growth (*e.g.*, oxygen, or favorable temperature), and, *second*, by observing whether the place of response corresponds with the place where growth is occurring. The first the student may readily plan for himself (with suggestions from DETMER, 448) and the second may be arranged thus:

EXPERIMENT. Place a strong-growing seed (*e.g.*, Horse Bean) in a wire-and-sphagnum cage and, when the root is 1–2 cm. long,

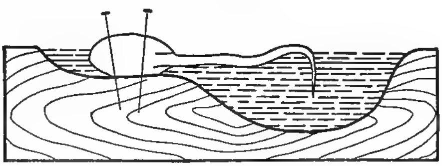


FIG. 66.—CONVENIENT ARRANGEMENT FOR SACHS' GEOTROPIC FORCE EXPERIMENT; $\times \frac{1}{2}$.

mark it along one side by the roller space-marker (page 197); then place it horizontal, marks upward, in a suitable moist-chamber, and note the relation between the growing and the responding parts.

Select a potted plant with slender-growing upright stem, mark it with the roller space-marker, place it with stem horizontal, marks underneath, and note effect of the response upon position of the marks.

The localization of response here shown suggests an inquiry as to whether this is confined to growing tips and may not occur elsewhere, a question which can partly be answered thus:

SUGGESTED EXPERIMENT. Select a growing grass-stem, or the creeping greenhouse Wandering Jew, and cut out a piece some 10 cm. long with a node in its middle. Place the lower end in water, and lay it horizontally, with the node and upper end free, which can be accomplished by thrusting it into a vial filled with wet sphagnum, or else, and better, by the arrangement of figure 67, with the pins on the left of the node omitted. Give favorable conditions for growth, preferably in a moist-chamber, and observe the resultant effects.

Observation of this experiment is likely to suggest a question as to the result in cases where all conditions are favorable for a response, but this is forcibly prevented. It may be tested as follows:

SUGGESTED EXPERIMENT. Prepare a piece of grass-stem as for the preceding experiment, but fasten it throughout horizontally in a way to keep the lower end wet, and to leave the node free; this may be accomplished by use of a block arranged as shown by figure 67, to which the stem is attached by crossed pins. Leave it for a day or two under favorable conditions for growth, preferably in a moist-chamber, then carefully observe the node.

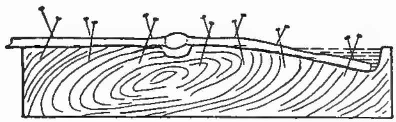


FIG. 67.—ARRANGEMENT FOR STUDY OF GEOTROPISM OF NODES; × ½.

Select a potted plant with an actively growing vertical stem, and by loops of thread tie this, quite to its tip, to a glass rod thrust into the earth. Place it horizontally for a few hours, then quickly cut the threads, and note the result.

The foregoing studies help to explain *the mechanics of geotropic responses*, upon which the student should extend his knowledge by study of the literature. He should include here, also, an inquiry into the effects produced upon the geotropic response by external conditions. It remains now to consider the exact way in which gravitation produces these results. Observing that the responses are localized in somewhat definite areas, one of the first questions to be settled is whether gravitation produces its effect directly upon the responding parts, or whether it may operate through other parts. The experimental testing of this matter presents so much difficulty, that the student may be obliged to turn for answer to the literature; but it can be answered thus:

SUGGESTED EXPERIMENT. Selecting the root as the part most practicable for experiment, and noting that its tip contains two prominent areas, the growing point and the elongating (which is the responding) zone, we proceed to test the possibility that gravitation may affect the former rather than the latter directly. This can be done by DARWIN'S method of cutting away the tip, leaving the elongating zone, and noting whether this zone can then respond geotropically; but as the method produces a profound functional change, a much better way is CZAPEK'S. In this the roots are made to grow into bent tubes of such construction that the tip and elongating zones may be bent at right angles to one another so that one at a time may be exposed to one-sided action of gravitation. Unfortunately this beautiful experiment is difficult, but directions are accessible in DARWIN and ACTON, 174. Compare also PFEFFER in Annals of Botany, **8**, 1894, 317, and in CZAPEK's own papers in Jahrbücher für wissenschaftliche Botanik, **27**, 1895, 243, and **35**, 1900, 313.

In connection with this experiment the student should inform himself as to our knowledge of the method of conduction of stimuli from cell to cell, including the possible existence of conducting structures.

The facts that plant parts grow away from and at various angles to gravitation as readily as towards it, that, as shown by the just-preceding experiment, the responding zone need not be the part which gravitation affects in causing the response, and that a response may occur long after the stimulus has ceased to act, all combine to prove that gravitation in its relation to the geotropic response does not act directly, that is, through weight of the parts. Nor are the phenomena by any means those which are characteristic of a tonic relation (page 70). There remains only one possibility, that gravitation is acting simply as a guide or signal of direction, that is, as a signal stimulus, and all the facts combine to sustain this conclusion. This leads to a further problem, precisely how, that is, by what physical method, the gravitation stimulus is perceived, or impresses itself, upon the sensitive protoplasm of the plant. As a first step toward solving this we ask whether there is any way by which gravitation can be replaced by some other force of equal power whose effects we can observe. Fortunately we have such a force in centrifugal force, and hence this problem:

What effect is produced upon the directions of young growing parts if gravitation is replaced by centrifugal force?

EXPERIMENT. To the margin of the disc of a centrifuge attach strong germinating seeds (*e.g.*, Corn, Horse Beans); arrange to keep them revolving rapidly in a vertical plane in a suitable moist-chamber, and observe the resultant positions of the young parts.

CENTRIFUGES. These must be of such construction as to revolve rapidly enough to give the seeds a centrifugal throw of some grams, and yet not so rapidly as to introduce purely mechanical bendings of the young parts. The method of determining the speed and force is given by PFEFFER, "Physiology," 3, 170. The first to use them was KNIGHT, over a century ago. The modern forms are usually driven by electric power, either by a primary battery, as in the excellent form described and figured by ARTHUR and supplied among his apparatus (Botanical Gazette, **22**, 1896, 463), or by a continuous power-current, as in NEWCOMBE's very perfect form recently described and figured (Botanical Gazette, **38**, 1904, 427). The standard electrical centrifuges sold for dairy and medical purposes (and of which the Bausch & Lomb Optical Company issue a special catalogue) are no doubt readily adaptable to this use, as PFEFFER has noted ("Physiology," 3, 170). A form driven by a hot-air motor has been described by HANSEN (Flora, **84**, 1897, 352). Much simpler forms may be adapted, driven by a water-current from a tap, as described by STEVENS (Botanical Gazette, **20**, 1895, 89), by DETMER, 462, and by various others later, including one by OSTERHOUT, 93, the latter's instrument being supplied ready for use by the Cambridge Botanical Supply Company.

Since centrifugal force undoubtedly results in throwing the heavier particles contained in a cell away from the center of rotation, thus producing a condition of one-directioned strain in the protoplasm, and since gravitation certainly produces the same effect, it seems probable that it is this condition of strain, through the downward pulling of the heavier cell contents, which gives the line of direction to the protoplasm which thus "perceives" the stimulus.

It is always important if a physiological process can be grounded definitely in chemistry or physics, and a good case of this is found in CZAPEK'S chemical test of a geotropically stimulated root, on which the student should now inform himself.

We come finally to consider geotropism from the ecological point of view, and ask why it is so extensively used as a guide by the parts of plants. In this connection it is to be noted that no direct value of geotropism to any function of the plant has been found, and that moreover it may even lead the parts into fatal positions, as is shown by the following:

SUGGESTED EXPERIMENT.—Prepare a trough of paraffined wire netting, and place on its bottom some soaked seeds. Then fill the trough with earth, hang it up in a moist-chamber, keep the soil moist, and note the result.

Our consideration of geotropism has shown that gravitation acts not mechanically, but as a signal stimulus, that it is perceived at one place while response takes place at another, that responses can be positive, negative, or intermediate to the same stimulus, and that the responses are such as to bring the parts as a rule into positions favorable for their functions, though under exceptional conditions they may bring the parts into unfavorable, or even fatal, positions. Since these features are true for one form of irritability, they may be true for others, a matter whose importance will be evident on further study.

(*b*) *Phototropism.*

The most familiar example, no doubt, of irritable responses of plants to external influences is the turning of green tissues towards light (phototropism or heliotropism). A logical beginning of its study is this problem:

What are the facts in the turning of green parts towards light?

EXPERIMENT. In the two compartments of a demonstration heliotropism chamber, place plants of Garden Nasturtium or other slender-petioled growing plant (or young seedlings), one in fixed position and the other revolving with axis vertical on a klinostat. Give strong one-sided light, and observe the resultant leaf-and-stem positions.

HELIOTROPISM CHAMBERS. A variety of forms exist, adapted to special phases of the study, all, however, being designed to keep the plant under healthful conditions in a dark chamber to which light can be admitted under control (compare MACDOUGAL, 129, who gives a form ventilated by aid of an aspirator, DETMER, 468, and the color chamber of SACHS in his Gesammelte Abhandlungen, 1, 299). For demonstration purposes, however, I have found very excellent a box, black inside, white outside, divided by a partition into two chambers each about 20×20 cm. square and 40 cm. high, open on one side. The light is then wholly one-sided, and can be further regulated as to amount and direction by a covering of black-and-white paper glued in place over the open side. A handle on top facilitates its ready transport.

The student should carefully note in this experiment the differences in the positions of stems and leaves, which will suggest in turn this question:

What response is made by roots to light?

Experiment. In a simple water-culture vessel (page 116) germinate radish or other convenient small seeds, and keep in dark until the roots and stems are 1 to 2 cm. long. Then place the vessel in a chamber with light coming wholly from one side through a space about its own size, and note the responses of the roots.

It will be noticed at once that the effects observed in this experiment are complicated by the action of geotropism, thus, incidentally, giving an excellent opportunity to observe the effect where two stimuli are acting at the same time, but from different directions. But for our present purpose we prefer the light alone, and gravity may be eliminated by the following:

Suggested Experiment. Germinate 4 or 5 seeds (Corn, Oats, Barley, Beans) upon corks on the margin of a klinostat disc revolving in a vertical plane (Fig. 60); let light fall upon them parallel with the axis of the instrument, and note positions of stems and roots.

Turning now to the location of the response in relation to the reception of the stimulus, we inquire:

Is the phototropic stimulus received at the place of actual response?

Experiment. Fill a small flower-pot with earth to the brim (so this will cast no shadow); sow in it a dozen Oats, and keep in darkness until these appear about 1 cm. above the surface. Prepare from tin-foil six very tiny cylindro-conical caps 5 mm. long and just large enough to fit snugly over the tips of the young shoots (they may be modelled on the lead of a lead-pencil); fit them upon half of the shoots (where they will cover the tips, but not the growth zone), leaving the others exposed; then place the pot in one-sided light, and note results.

In this connection the student should inform himself as to our knowledge of *the place and method of reception of the light stimulus* in other parts, notably in leaves, on which he should note Haberlandt's recent theory of the function of the swollen epidermal cells. Also, he should inquire as to *the quantitative relation existing between stimulus and response, the application of Weber's law, and the relations of the energy supplied by the stimulus to that causing the response.*

Every student, in working with light effects upon plants, will recall that sunlight is a very composite source of energy, and will wish to determine:

Which of the rays in white light induce phototropic curvatures?

EXPERIMENT. Prepare 4 dark chambers, each some 15 cm. square; in the middle of one side of each of them, leave an opening 4 cm. in diameter, and cover this outside with a Soyka flask, which is attached so that no leakage of light can occur (by black gummed paper); fill the flasks with, respectively, red, green, blue, and white (control) liquids made up spectroscopically pure according to the directions earlier given (page 111). Place in each chamber a small pot containing Oats or Barley, germinated in darkness to about 2 cm. high; give them all equal exposures, and observe at intervals to ascertain under which the phototropic response is quickest and most pronounced.

The ideal way to solve this problem is through use of a spectrum thrown laterally upon the young seedlings, but the practical experimental difficulties are very great.

Special phases of phototropic irritability, susceptible of experimental study, are the movements of chlorophyl grains, and the locomotive movements of some free-swimming organisms (phototaxis).

All of the responses to light so far considered are to light from one direction, but there are cases of another character, of which the sleep movements of Clover, Oxalis, or Mimosa leaflets are typical, in which the stimulus is not unilateral, but diffused, and the responses are said to be photonastic instead of phototropic. These sleep movements, indeed, are of such frequency that the sleep response has been given a special name, nyctitropism (though it should be nyctinasty), and they are readily accessible to experiment.

SUGGESTED EXPERIMENT. Take at midday a potted Oxalis or Mimosa with expanded leaflets, and enclose it in a ventilated dark chamber; then note any change in the leaflets, and the rapidity of the effect. Also ascertain whether there is any difference in the response at different hours of the day.

The foregoing experiments will give the student an idea of the essential facts of phototropism, and he should now bind and weld his personally acquired knowledge into a broader and deeper comprehension by study of the literature.

(c) *Hydrotropism.*

The tendency of roots to seek wet places is a familiar phenomenon of the gardens and a manifestation of hydrotropism. For its study we ask first:

What are the facts in a typical case of the turning of roots towards moisture?

This may be tested by so arranging roots growing geotropically downward that they will be deflected, if sensitive, by a moist surface near them.

Experiment. Select two clean porous flower-pots of 5-inch or 6-inch size; stopper both ends (Fig. 68), and by aid of a round file

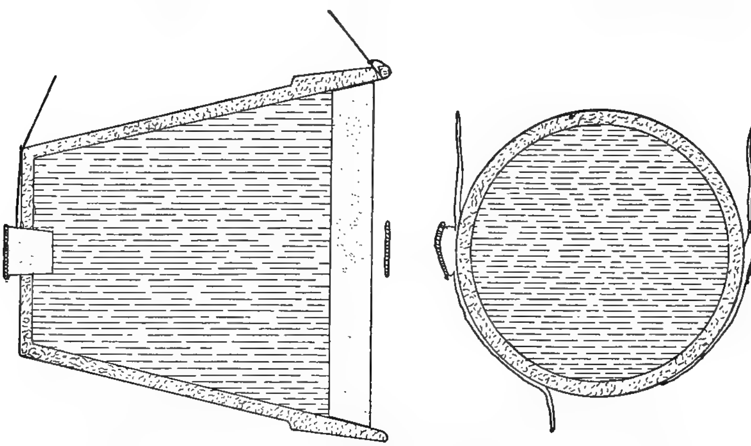


Fig. 68.—Arrangement for the study of hydrotropism; $\times \frac{4}{9}$.
On the right is a cross-section of the flower-pot about its middle. It may be filled with saturated sphagnum instead of with water.

worked in a carpenter's brace, bore a small hole near the upper margin; tie lengthwise around, as in the figure, a double strip of cheesecloth, between which and the pot place a number of soaked seeds; fill the pots with water (or pack them with very wet sphagnum) and suspend them in bell jars, one of which is to be kept with saturated atmosphere, while the other is to be given moderate humidity. Compare the positions of the roots in the two, and note approximately the critical angle at which the roots leave the moist surface.

Other Methods. The arrangement described above serves admirably for this purpose, and permits very effective demonstration, all the better for the great contrast in color between roots and pot. It has the great advantage of providing a constantly increasing curvature of surface, permitting the critical angle, where the influence of geotropism overpowers that of hydrotropism, to be determined; and, moreover, by use of different sizes of pots, it allows surfaces of any desired curvature to be obtained. If a pot be filled with sphagnum kept saturated, and if seeds be planted around its edge with their young roots projecting over, these will follow the sloping surface of the pot very beautifully. Instead of the pots one may use the low cylinders formed by Zurich germinators, two of which placed edge to edge and filled with wet sphagnum form an excellent arrangement, as described in the first edition of this book (page 130). The usual way of demonstrating hydrotropism is that introduced by SACHS, consisting of an inclined trough of wire netting filled with sawdust or soil containing the seeds, the roots of which issue from the netting, but keep close to the moist slope. Another, but inferior, way commonly recommended lies in the use of a funnel containing soil or sawdust, and covered with filter-paper kept wet by water in a supporting bottle, the seedlings being started with their roots over the edge. In the use of all of these methods, the regulation of the humidity of the surrounding air is very important; for if this is kept at saturation, the hydrotropism is neutralized and only geotropism prevails, while if too dry the roots die at the tips. The humidity can best be regulated by use of a bell jar open at the top; this can be raised upon blocks, or other support, a little from the table until the right amount of humidity is found. A method of showing hydrotropism of roots in the soil is given by OSTERHOUT, 96.

The results of this experiment upon roots will at once suggest the correlative inquiry as to the hydrotropism of stems, upon which matter the student may now be left to devise and carry out a good experimental test for himself, if, indeed, the preceding experiment does not settle this point. And he should now complete his knowledge of hydrotropism by aid of the literature.

(*d*) *Thigmotropism.*

Responses to contact, or to mechanical shocks of various kinds, are fairly familiar in plants. The student should now plan (with some suggestions from DETMER and from DARWIN and ACTON) and carry out a series of experiments and observations directed to show the facts as to:

(*a*) The responses of tendrils (and some twining and parasitic stems) to contact (Haptotropism).

(*b*) The responses of the Sensitive plants (Mimosa and others) to various kinds of stimuli, also of Venus' Flytrap and Sundew.

(*c*) The responses of stamens of Barberry, Opuntia, etc., and the stigmas of Mimulus.

Upon this basis the student should work up this subject through the literature, giving somewhat special attention to the mechanism of the responses, and to acclimatization to stimuli. Here also he may best consider *the results following a summation of stimuli, a competition of stimuli, and a substitution of stimuli acting upon the same part.* And this will lead naturally to a study of *the essential nature of a stimulus*, with the characteristics it must present in order to act as a stimulus at all; and this in turn will involve the problem of *the relation between irritability on the one hand, and sensation with reflex action on the other.*

(*e*) *Chemotropism.*

Of very much importance, even though not very conspicuous, are responses to chemical substances. For the most part the experimental study is somewhat difficult, though not impracticable, and the student should follow, experimentally as far as possible, and then through the literature, the following matters:

(*a*) Chemotaxis of the antherozoids of Ferns and of Bacteria (compare PFEFFER'S fine experimental methods, and BULLER, Annals of Botany, **14**, 1900, 543).

(*b*) Chemotropism (with aerotropism) of pollen tubes, hyphæ of Fungi, young roots (compare NEWCOMBE and RHODES in Botanical Gazette, **37**, 1904, 23, and LILIENFELD in Beihefte zum botanischen Centralblatt, **19**, 1906, 131).

(*c*) Gall and other structural formations; also fruit formation after fertilization.

(*d*) Regulation of the growth of petioles of water-plants, upon which a striking and valuable experiment is practicable.

SUGGESTED EXPERIMENT. Select a typical water-plant of the floating-leaf type (*e.g.*, *Limnocharis*, Water Poppy) growing in a tank in a few inches of water. Suspend over the tank a large glass tube, some 4 or 5 cm. internal diameter and a meter long, closed at the upper end and nearly filled with water. Into this tube guide a young developing leaf. Beside this tube place another also closed above, but filled with air; depress it to near the bottom of the tank, and guide into it a young leaf. Thus the leaves are supplied with three different water-levels. Compare the relative growths of the petioles of the various leaves, and observe the cellular basis in the petioles. Also with other leaves replace the air in both tubes by nitrogen or hydrogen, and observe the result upon petioles. (Upon this illuminating experiment compare KARSTEN in Botanische Zeitung, **49**, 1888, 565.)

(*f*) *Thermotropism.*

True thermotropic movements are not common, though they may be demonstrated experimentally (DETMER, 478), but there are some prominent thermonastic responses, as in the opening and closing of some flowers, and in the alteration of position of Rhododendron and other leaves. These are quite distinct in kind from Thermometric movements, one of which I have myself described in Annals of Botany, **18**, 1904, 631. All of these matters, however, are difficult of experiment, but the student should follow them through the literature.

(*g*) *Other Tropisms.*

In addition to the more important forms of irritability above mentioned, there are many others of minor importance, some of which are only modifications or substitutions of those already described. The more important are: *Traumotropism*, or response to injury, which may be demonstrated by methods given by DARWIN in his "Power of Movement in Plants" (compare also REED, Journal of Applied Microscopy, **6**, 2466, and SPALDING's discussion in Annals of Botany, **8**, 1894, 423); *Rheotropism*, response to direction of water-currents, which is proving simply a phase of Thigmotropism (compare NEWCOMBE in Botanical Gazette, **33**, 1902, 177); *Electrotropism* and *Galvanotropism*, or response to electrical currents, of some special interest, since their responses could not have been adaptively acquired and must be simply coincidental; responses to mechanical strains, resulting in development of strengthening tissues; responses in varying thickness of cuticle, etc., in desert plants in relation to degrees of dryness; *autotropic* responses, or those determined by form conditions of the organism itself.

Finally the student should make a classification of the different known forms of Irritability, giving their names, physical character of the stimulus, mode of its impress upon the protoplasm, place of the same, directions of the known responses, nature of the responding mechanism, and ecological significance.

And he should close with a brief exposition, or even definition, of Irritability.

Literature of Irritability. The literature of this important subject is fully summarized in Volume III of Pfeffer's "Physiology," which brings the subject down to so recent a date as hardly to leave anything later to mention, and there is the usual good synopsis in Jost. There is also a very suggestive treatment of the subject by Verworn, though not without some flaws so far as plants are concerned. Some notes on the latest papers are in the Botanical Gazette, **43**, 1907, 218, 226. Pfeffer has given a very clear popular treatment of the several subjects in Nature, **49**, 1894, 586, while a very clear scientific-popular treatment of the whole subject, in a series of articles by F. Darwin, is in the New Phytologist, **5**, 1906, and **6**, 1907.

12. ADAPTATION.

Organisms exhibit not only individual adjustments to their surroundings through irritable responses, but also a race adjustment, or adaptation, both in structure and habit, to the general conditions of the surroundings. This is fixed in heredity, and is generally supposed to have been acquired by the natural selection of variations (or mutations). It has three great leading phases: (*a*) development of structure (including form, size, color, texture); (*b*) locomotion; (*c*) protection. Some phases have already been noted in this book, but as a whole its study is a distinct department of investigation, properly comprehended under Ecology.

PART III.

MANIPULATION AND TABLES IMPORTANT IN PLANT PHYSIOLOGY.

MANIPULATION.

AIR. *Aeration* apparatus, aspirator, exhaust, blast; see page 48. Aeration of closed chambers is usually effective if once daily the old air is blown out (by a puff from the lips). *Or* a dish of caustic-potash solution in the chamber will absorb the noxious constituent, carbon dioxide.

Drying may be effected as described on page 158.

Tightness may be tested by the apparatus described on page 51.

BLUE-PRINTS. *Drawings* in black and white may be made from them thus: Go over the dry blue-print, along the desired lines, with waterproof India ink. When dry lay the drawing in a very weak solution of common soda (sal soda). After the blue is gone rinse the print cautiously in water, and then if it shows any yellow stain, put it for a few moments in dilute hydrochloric or sulphuric acid. Wash it in water and dry on a glass plate. Of course one should experiment first upon a valueless print. The ink must be pure and the pen clean.

BRASS. *Corrosion* may be removed by immersing the part for a few minutes in concentrated hydrochloric acid. It should then be washed thoroughly in running water and dried over a flame. Finally if touched while hot with a bit of paraffin, this will spread and protect the clean brass from further corrosion.

CARBON DIOXIDE. *Absorption and Analysis.* See page 98.

Generators. See page 52.

CHEMICALS. *Purity* is safeguarded by a rigid rule never to return any chemical, even if not used, to its bottle.

CONNECTIONS. *Glass tubing of same size.* Bring the ends (preferably ground square across, or at least smoothed in the flame) together in the middle of a piece of tightly clasping rubber tubing. Where perfect air-tightness is required, the rubber may well be lightly glued, by fish-glue, to the glass, or else may be wired by a single turn of small copper wire twisted up tightly by nippers. If there is pressure from within, it may be resisted by several turns of the inelastic and very adhesive electricians' (or tire) tape.

Glass tubing of different sizes. If only a little different, build up the smaller with thin rubber tubing to the size of the larger, and proceed as above; if one will slip inside the other, coat 1 to 2 cm. of the smaller with sealing-wax and slip inside the larger, which is also heated. When cold this forms a very good joint, though it is not permanently tight.

Glass tubing to stems. Proceed precisely as above described, except that, if the stem is delicate, the wire must not be used, since it will cut or compress the tissues; and string or a stretched rubber band must be employed. If the surface of the stem is channelled or otherwise irregular, some firm wax (see below) should be used between stem and rubber.

Stems to bored glass plates and necks of bottles. Use a bored-and-split soft-rubber stopper, with firm wax (described below). *Or* seal with mercury by a method given in MacDougal, 229. *Or* use gelatin by Devaux's method (Darwin and Acton, 108).

CORKS. *Air-tightness* is secured by soaking them in melted hard paraffin (in a water-bath), or, less efficiently, by smearing with vaseline.

Boring for tubes, etc., is effected by standard cork-borers, helped by round files.

Improvement is made in every way through softening them by a cork-presser, of which the rotary form is best.

Insertion into nearly full bottles may be effected by interposing between cork and glass a thread or a wire (finally withdrawn) along which the air may escape.

DRAWINGS. See Blue-prints.

EVAPORATION. *Prevention* is possible thus: If a free water-surface, cover with a film of oil, preferably machine-oil. If a moist surface, work over it a thin coating of modelling-wax. For other methods see page 173.

GERMINATING SEEDS. See page 210.

GLASS BOTTLES. *Cleaning* from tenacious sediment is effected by vigorous shaking with water and small shot, the latter kept stored in a bottle for the purpose.

Boring holes of any desired size may be accomplished by use of the end of a round file rotated with a carpenter's brace and kept wet with turpentine and camphor (12 weights of turpentine to 1 of camphor, freshly prepared). Or emery flour and camphor are said to be excellent, and better yet is dentists' carborundum. Instead of the file 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.

Cutting across. See under Glass Tubing, Cutting.

Labelling. See Labels.

GLASS FILAMENTS. May best be made from Glass Tubing, Capillary, which see.

GLASS TUBING. On manipulation there is a very useful little handbook, "The Methods of Glass-blowing," by W. A. Shenstone (London, Longmans, Green, & Co., 1902).

Bending. The smoothest bends can be made by heating the tube, which is kept revolving, in a luminous flame of the fish-tail form (or in the Bunsen wing-top), and the bend is better if the softened glass is allowed to settle by its own weight. The tube is held lengthwise in the flame for very gradual bends, and obliquely for shorter turns. For special curves, etc., the hot glass may be moulded by a cold iron.

Capillary. Hold the smallest available glass tubing in the Bunsen 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. To keep the capillaries straight, pull up and down, not horizontally.

Calibrating. See Graduating, below.

Cutting. If under 1 cm. diameter, file a nick on the desired line of cut; then, grasping the tube firmly with fingers each side of the nick, which is to be turned away from the worker, pull the ends of the tube apart, at the same time snapping it sharply outward in the middle, when it will break across. If somewhat over 1 cm. diameter, a deeper nick with greater exertion will still permit it to be broken in this way. But if much larger, or if a vial or bottle is to be cut across, another method must be used, such as the following: File a groove along the entire line of the desired cut, making it deeper in one place; then, pulling the two ends gently apart, bring the nick into the tip of a very fine flame, when the glass will crack along the line of the groove. Another method, especially efficient with large bottles, is this: File a nick upon the desired line of cut, making it taper up at both ends; wrap around the glass two long strips of wet filter-paper parallel with the desired line of separation, but 1 mm. from it in thin glass and more in thicker; then bring the nick into the tip of a fine flame, when it will crack cleanly along the desired line. Also, a very hot iron point led along the line to be broken is said to be effective. Also, for large tubing a very efficient special cutter may be purchased. If a very short end, too short to be grasped firmly in the hand, is to be cut from a tube, file a nick in the usual way, then rest the tube with the nick (which is to be upwards) over the edge of a file. A smart blow on the small end with a light metal object, such as a key, will then break the end cleanly off.

Graduating. If only regular interval marks are wanted, these may be well applied by use of the wheel-marker for stems, earlier described (page 197). If measured intervals are needed, they may be applied with waterproof India ink by use of a ruler and thread-marker made from a thread stretched by a small wire bow. *Or*, and for most purposes best of all, a pasteboard or wooden millimeter scale is wired to the tube. *Or* tubes of injured thermometers (and, better yet, the scales of milk-glass scale thermometers) wired to the tubes are excellent. To make the graduation permanent, mark the lines with a writing diamond, or with a fine file, and rub into the marks some white lead, or some plaster of Paris containing a soluble color, such as carmine, or even waterproof India ink.

It is not possible to manufacture glass tubing of uniform bore, and hence for very exact work, especially if short lengths of tubing are involved (as when an air column is compressed under several atmospheres), it is necessary to *calibrate* it, that is, to determine and mark the error due to the inequalities. It is accomplished by introducing a short thread of mercury into the tube, pushing it along from point to point, measuring its length at each stage, and marking this upon the tube. If the stage with sliding microscope employed for this purpose in physics is not available, a fair substitute is found in the use of an ocular micrometer, the gauge being moved over the stage, while the thread of mercury may be forced along by use of a pipette bulb on one end of the gauge, or, better, by an adaptation of the screw arrangement found in a Reichert thermo-regulator.

Sealing. If held, sloping downwards, in the hottest part (the tip of the inner cone) of a Bunsen flame, and slowly revolved, small tubing will seal itself together perfectly. Or, if the precise shape does not matter, it may be held in a flame until it softens, when, if drawn apart, it will seal itself with a conical tip, and this is the best method with tubing above 6 mm. diameter. An open capillary point may be sealed by a very tiny flame, even of a match.

Smoothing cut ends. Hold the rough parts in the Bunsen flame until they fuse smooth. Or grind them upon an emery-wheel (page 48). Small tubing may be thrust at once into the flame, but large tubing must be brought into it cautiously. The rough ends of fine capillary tubing (which may be cut across with scissors) may be smoothed by careful rubbing on an oilstone, or on a piece of ground glass, or by cautious fusing.

GROUND STOPPERS, when stuck, may be loosened thus: If the top of the stopper is flat, place this in the hinge-crack of a door partly closed upon it, turn the bottle gently, when the stopper will probably loosen. *Or*, holding the bottle suspended by the stopper a half inch above the table, tap the stopper with a wooden hammer-handle or equivalent, when the bottle will usually drop from the stopper. *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, when the heat swells the neck and usually loosens the stopper. *Or* revolve the neck of the bottle quickly in the Bunsen flame, when the swelling of the neck will loosen the stopper. It is also said that the friction of a silk handkerchief rapidly drawn across and around the neck of the bottle will warm and swell it enough to loosen the stopper.

HEAT. *Separation from light* may be effected by interposition of an alum bath; but for most purposes running water from a tap carried through a flat-sided chamber, *e.g.*, a gas-chamber (page 72), is more convenient, especially where the microscope is concerned.

HOODS. If used for darkening plants, they should be black inside, but white outside in order to prevent excessive heating. It is well to have a supply always ready, made of black sateen and white cambric, of a size to fit the standard bell jars. A paper, black on one side and white on the other, is obtainable from supply companies. Gray felt paper makes a good compromise material.

HYDROSTATS. *Water-levels* may be kept constant in various ways. The Mariotte device consists of a water-filled inverted vessel with its outlet just above the level to be maintained; as the water-level falls air is admitted to the vessel, thus allowing water to escape and raise the level. An inverted bottle, with a grooved cork projecting just so far from the neck as the desired depth of the water, serves very well. *Or* the bottle may be placed at a distance, with a glass tube extending to the desired water-surface. Different arrangements are figured in the first edition of this book, pages 108, 109.

For keeping a water-supply constant in soil, there is an arrangement by Krutitzky (see Burgerstein, "Die Transpiration," 22), while a recent and efficient arrangement is Livingston's (see page 176 of this book).

HYGROSCOPICITY. For overcoming this in threads see page 204.

JOINTS. See Connections.

LABELS. *For bottles.* If glass-stoppered, write with pencil on the stopper, and it will show through the neck. *Or* a colored paraffin pencil made expressly for writing on glass may be used. *Or* the inscription may be written in waterproof India ink, and when dry covered with Canada balsam. *Or* a paper label may be applied, and afterwards coated thinly with moderately hard paraffin, applied very hot, said to be a most efficient and otherwise satisfactory method. *Or* a writing diamond, costing about $3.00, is very useful for some purposes. For temporary labelling, druggists' tag labels are most useful.

MERCURY. *Container.* See page 50.

Cleaning may be accomplished sufficiently for most physiological purposes by thorough washing in running water carried down through it by a glass tube. *Or* a continued current of air through it cleans it well. *Or* it may be allowed to drop through a column of weak nitric acid and subsequently washed. Compare DETMER, 42, 43.

Experimenting with mercury should always be done over an indurated fiber saucer, which will catch the leakage.

OXYGEN. *Absorption and Analysis.* See page 103.

Generators. As described in works on Elementary Chemistry. But it is most convenient to obtain it from the cylinders supplied commercially.

READING GRADUATIONS accurately on thermometers, etc. For this purpose a special magnifier sliding on the tube is supplied by LEITZ, and another by GRIFFIN & Sons of Kingsway, London. These instruments could readily be adapted to other accurate readings.

REGISTERING. For various purposes suitable instruments are described earlier at pages 145, 178, 181, 200, and most of them, especially the Auxograph (page 203), may readily be adapted to other uses.

Gauges or manometers may to some extent be made registering by dropping on the liquid some very fine cork dust, a line of which will be left at the highest point reached.

Pens for the purpose may be made from glass tubing, or are sold in good forms by the Cambridge (England) Scientific Instrument Company.

Smoked paper is sometimes used with pointers scratching upon it. The paper is smoked by turpentine burning in a spirit-lamp, and the records may be made permanent through spraying, by an atomizer, with weak gum-arabic solution.

RUBBER. *Boring or cutting* is always easier if rubber and instrument are kept wet with water, or, better, with caustic potash.

Preserving is facilitated by darkness, and in some cases by immersion under water.

Stoppers with holes may best be sealed by pieces of glass rod smoothed in the flame, or by pieces of sealed glass tubing.

SIPHONS. *Starting without suction.* Use a rubber tube and immerse it in the liquid, allowing it to fill; pinch together one end so the air cannot enter; lift this end out over the edge of the vessel and let it fall below the inner level; allow the pinched end to open, when the flow will start. If of glass fill it with water and hold the fingers over the two ends until it is in position.

STERILIZING. Best done by steam in a suitable instrument. See page 48.

STOP-COCKS. Should always have their cores attached by string to their tubes, as otherwise they are liable to fall and break. They should be kept thinly lubricated by a special wax supplied for the purpose.

TRANSPORTING EXPERIMENTS for demonstration purposes. For this purpose the experiment should be set up on a tray (or a large indurated fiber saucer) provided with handles. For this purpose I have devised a special portable support or clamp stand, which is shown in figures 62, 63, and is supplied among my normal apparatus. It proves very convenient in use.

VACUUM. This, or an approach to it, may readily be made evident in a small chamber by use of a small U-shaped tube of glass tubing sealed at the end of one arm which is filled with mercury, the other arm being open and only partly filled; a vacuum is marked by the fall of the mercury to the same level in both arms.

WAXES. Needed for making tight connections and some other uses. A very excellent kind is a mixture of beeswax and vaseline, the proportions being varied according to weather or use. It is well to have vials of various combinations always ready. For some purposes cocoa-butter, which melts at about 33°, is valuable (see page 166).

WILTING. May be checked by method given on page 169.

TABLES.

MEASURES AND WEIGHTS.

CONVERSION TABLES.

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 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. $4\frac{3}{5}$ 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

COMBINING WEIGHTS OF THE ELEMENTS MOST IMPORTANT IN PLANT PHYSIOLOGY.

Barium	Ba	137.4
Calcium	Ca	40.1
Carbon	C	12.
Chlorine	Cl	35.45
Hydrogen	H	1.008
Iodine	I	126.97
Iron	Fe	55.9
Magnesium	Mg	24.36
Mercury	Hg	200.
Nitrogen	N	14.04
Oxygen	O	16.
Phosphorus	P	31.
Potassium	K	39.15
Silicon	Si	28.4
Sodium	Na	23.05
Sulphur	S	32.06

TEMPERATURES.

Conversion Table; Centigrade to Fahrenheit and vice versa.

Centigrade to Fahrenheit, multiply by $\frac{9}{5}$ and add 32. Fahrenheit to Centigrade, subtract 32 and multiply by $\frac{5}{9}$.

C.	F.	C.	F.	C.	F.	C.	F.	C.	F.
100	212	45	113	25	77	5	41	−15	5
93.33	200	44.44	112	24.44	76	4.44	40	−15.55	4
90	194	44	111.2	24	75.2	4	39.2	−16	3.2
87.78	190	43.89	111	23.89	75	3.89	39	−16.11	3
82.22	180	43.33	110	23.33	74	3.33	38	−16.67	2
80	176	43	109.4	23	73.4	3	37.4	−17	1.4
76.67	170	42.78	109	22.78	73	2.78	37	−17.22	1
71.11	160	42.22	108	22.22	72	2.22	36	−17.78	0
70	158	42	107.6	22	71.6	2	35.6	−18	−0.4
65.56	150	41.67	107	21.67	71	1.67	35	−18.33	−1
65	149	41.11	106	21.11	70	1.11	34	−18.89	−2
62.78	145	41	105.8	21	69.8	1	33.8	−19	−2.20
61	141.8	40.55	105	20.55	69	0.55	33	−19.44	−3
60	140	40	104	20	68	0	32	−20	−4
59.44	139	39.44	103	19.44	67	−0.55	31	−20.56	−5
59	138.2	39	102.2	19	66.2	−1	30.2	−21	−5.80
58.89	138	38.89	102	18.89	66	−1.11	30	−21.11	−6
58.33	137	38.33	101	18.33	65	−1.67	29	−21.67	−7
58	136.4	38	100.4	18	64.4	−2	28.4	−22	−7.60
57.78	136	37.78	100	17.78	64	−2.22	28	−22.28	−8
57.22	135	37.22	99	17.22	63	−2.78	27	−22.78	−9
57	134.6	37	98.6	17	62.6	−3	26.6	−23	−9.40
56.67	134	36.67	98	16.67	62	−3.33	26	−23.33	−10
56.11	133	36.11	97	16.11	61	−3.89	25	−23.89	−11
56	132.8	36	96.8	16	60.8	−4	24.8	−24	−11.20
55.55	132	35.55	96	15.55	60	−4.44	24	−24.44	−12
55	131	35	95	15	59	−5	23	−25	−13
54.44	130	34.44	94	14.44	58	−5.55	22	−25.56	−14
54	129.2	34	93.2	14	57.2	−6	21.2	−26	−14.80
53.89	129	33.89	93	13.89	57	−6.11	21	−26.11	−15
53.33	128	33.33	92	13.33	56	−6.67	20	−26.67	−16
53	127.4	33	91.4	13	55.4	−7	19.4	−27	−16.60
52.78	127	32.78	91	12.78	55	−7.22	19	−27.22	−17
52.22	126	32.22	90	12.22	54	−7.78	18	−27.78	−18
52	125.6	32	89.6	12	53.6	−8	17.6	−28	−18.40
51.67	125	31.67	89	11.67	53	−8.33	17	−28.33	−19
51.11	124	31.11	88	11.11	52	−8.89	16	−28.89	−20
51	123.8	31	87.8	11	51.8	−9	15.8	−29	−20.20
50.55	123	30.55	87	10.55	51	−9.44	15	−29.44	−21
50	122	30	86	10	50	−10	14	−30	−22
49.44	121	29.44	85	9.44	49	−10.56	13	−30.56	−23
49	120.2	29	84.2	9	48.2	−11	12.3	−31	−23.80
48.89	120	28.89	84	8.89	48	−11.11	12	−31.11	−24
48.33	119	28.33	83	8.33	47	−11.67	11	−31.67	−25
48	118.4	28	82.4	8	46.4	−12	10.4	34.44	−30
47.78	118	27.78	82	7.78	46	−12.22	10	−40	−40
47.22	117	27.22	81	7.22	45	−12.78	9	−45.56	−50
47	116.6	27	80.6	7	44.6	−13	8.6	−50	−58
46.67	116	26.67	80	6.67	44	−13.33	8	−73.33	−100
46.11	115	26.11	79	6.11	43	−13.89	7	−100	−148
46	114.8	26	78.8	6	42.8	−14	6.8	absolute zero	
45.55	114	25.55	78	5.55	42	−14.44	6	−273	−459

NAMES, ENGLISH AND BOTANICAL, OF THE PLANTS MOST USED IN EXPERIMENTAL PLANT PHYSIOLOGY.

Abutilon	*Abutilon striatum* ×
Barley	*Hordeum vulgare (sativum)*
Beans:	
Bush Bean	*Phaseolus vulgaris nanus*
Horse Bean	*Vicia Faba equina*
Lima Bean	*Phaseolus lunatus*
String Bean	*Phaseolus vulgaris*
Windsor Bean	*Vicia Faba*
Begonia	*Begonia coccinea*
Buckwheat	*Fagopyrum esculentum*
Castor Bean	*Ricinus communis*
Cineraria	*Cineraria cruenta*
Cestrum	*Cestrum elegans*
Coleus	*Coleus Blumei*
Corn	*Zea Mais*
English Ivy	*Hedera Helix*
Fuchsia	*Fuchsia speciosa*
Garden Nasturtium	*Tropæolum majus*
Geraniums:	
Horseshoe	*Pelargonium hortorum (zonale* ×)
Lady Washington	*Pelargonium domesticum*
Ivy-leaved	*Pelargonium peltatum*
German Ivy	*Senecio mikanioides*
Heliotrope	*Heliotropium peruvianum*
Impatiens	*Impatiens Sultani*
Jacobinia	*Jacobinia magnifica*
Marguerite	*Chrysanthemum frutescens*
Morning-glory	*Ipomœa purpurea*
Mustard	*Brassica alba*
Oats	*Avena sativa*
Oxalis	*Oxalis Bowiei*
Peas	*Pisum sativum*
Poinsettia	*Euphorbia pulcherrima*
Primroses:	
Obconica	*Primula obconica*
Chinese	*Primula sinensis*
Radish	*Raphanus sativus*
Rubber Plant	*Ficus elastica*
Salvia	*Salvia involucrata*
Senecio	*Senecio Petasitis*
Spiderwort	*Tradescantia virginiana*
Squash	*Curcubita Pepo*
Sunflower	*Helianthus annuus*
Tomato	*Lycopersicum esculentum (Solanum Lycopersicum)*
Wandering Jew	*Tradescantia zebrina*
Wheat	*Triticum sativum*
White Lupine	*Lupinus albus*

The most useful single plant for physiological experimentation is the Fuchsia, *Fuchsia speciosa.*

CONVENTIONAL CONSTANTS OF SOME OF THE PRINCIPAL PROCESSES OF PLANT PHYSIOLOGY.

(For the explanation of their derivation and use, see page 21.)

Growth (page 207).	Temperatures for plants commonly used in experiment: Minimum. $0°$–$5°$–$15°$ Optimum. $25°$–$30°$–$35°$ Maximum. $30°$–$40°$–$45°$
Photosynthesis (page 113).	Formula of the Photosynthate, $C_6H_{12}O_6$. Photosynthetic Equation, $6CO_2+6H_2O=C_6H_{12}O_6+6O_2$. Quantity of Photosynthate made in leaves: Out-of-doors, 0–**1**–2 *gm²h.* Greenhouse, 0–**.5**–1 *gm²h.* Synthesis of 1 gram of Photosynthate requires 1.5 g. (750 cc.) of carbon dioxide, which is all that is contained in 2500 liters of air, which is a column 1 meter square and 2.5 meters high. Energy of strong sunlight used, 1%. Energy stored is the reciprocal of that released in Respiration (see below).
Respiration (page 141).	Formula of material used (ultimately), $C_6H_{12}O_6$. Respiratory Equation, $C_6H_{12}O_6+6O_2=6CO_2+6H_2O$. Quantity of material used in Respiration: Leaves: $20°$ .12 (60 cc.) $30°$ .30 (150 cc.) $40°$ .65 (325 cc.) *gm²h.* Leaves 4. Buds 15. Seeds 30. } *gkh.* Energy developed from 1 gram Photosynthate equals 4000 calories. This is half the energy yielded by combustion of equal weight of best fuel.
Root Exudation and Pressure (page 157).	Exudation 1–**25**–300 cc. Pressure .3–**.9**–1.6 atmospheres.
Stomata (page 192).	Numbers in plants used commonly in experimentation: 0–**100**–500 per *mm²*, which is over 100 to the square millimeter, or ten million to the square meter. Size of Pore is 18×6 microns, giving an area of 100 square microns, which is $\frac{1}{100}$ part of the epidermal surface.
Transpiration (page 193).	In greenhouse plants { by day 15–**50**–250 *gm²h.*; by night 1–**10**–20 *gm²h.*; day and night together 1–**30**–250 *gm²h.*

AIR CONSTANTS.

<table>
<tr><td>Composition.</td><td>Oxygen. Varies from 20.906 to 20.938, with a mean at 20.922,—conventionally **21**.
Nitrogen. Varies somewhat with a mean at 79.049,—conventionally **79**.
Carbon dioxide. Varies from .0271 to .0323, with a mean at .0285,—conventionally **.03**.
Other materials. Traces.</td></tr>
<tr><td>Contraction and Expansion.</td><td>Perfectly dry air contracts and expands 1/273 of its volume for each degree Centigrade of fall or rise in temperature (1/459 for each degree Fahrenheit).</td></tr>
<tr><td>Pressure.</td><td>At Sea Level. (See under Barometric values.)
Mariotte's (also called Boyle's) Law, *viz.*, that pressure and volume in a gas are inversely proportional to one another, is true only for perfectly dry air.</td></tr>
<tr><td>Solubility in Water.</td><td>Pure Water absorbs from air at 760 mm. pressure the following percentages of its own bulk of
<table>
<tr><th>Oxygen.</th><th>Nitrogen.</th><th>Carbon Dioxide.</th><th>Total Air</th><th>At</th></tr>
<tr><td>.679</td><td>1.27</td><td>.04</td><td>1.989</td><td>10° C.</td></tr>
<tr><td>.625</td><td>1.167</td><td>.03</td><td>1.822</td><td>15°</td></tr>
<tr><td>.592</td><td>1.107</td><td>.02</td><td>1.719</td><td>29°</td></tr>
</table>
Hence at 15° pure water can absorb from air about the same absolute volume of carbon dioxide which the air itself holds, but at higher temperatures less, and at lower, more.
With increase of pressure these gases are absorbed exactly in ratio of the increase (Henry's Law).
Pure water can absorb from pure carbon dioxide at 15° and 760 mm. pressure, 100.2% of its own volume, from pure oxygen 2.989%, and from pure nitrogen 1.478%.</td></tr>
<tr><td>Rarefaction *vs.* Standard Volume.</td><td>Rarefied gas (over a column of mercury or water) may be reduced to standard volume, thus:

$$V=\frac{760-x}{760}\times v,$$

where V is desired volume, x is height of mercury in millimeters, and v is observed volume.
For water replace 760 by 10298.1.</td></tr>
<tr><td>Volume.</td><td>See Pressure, above.</td></tr>
<tr><td>Weight.</td><td>Weight in grams of 1 liter of
<table>
<tr><th>Oxygen.</th><th>Nitrogen.</th><th>Carbon Dioxide.</th><th>Air.</th><th>Hydrogen.</th></tr>
<tr><td>1.4295</td><td>1.2572</td><td>1.98</td><td>1.29327</td><td>.0900</td></tr>
</table></td></tr>
</table>

BAROMETRIC CONSTANTS.

	Inches	Millimeters
Comparison of English and Metric.	29 30 31 1. .1 .01	736.59 761.99 787.39 25.4 2.54 .254
Assumed Standard Height at Sea Level.	30 inches	760 millimeters
Pressure at Sea Level..	14.657 (conventionally **15**) pounds to 1 square inch	1.0333 (conventionally **1**) kilogram to 1 square centimeter

VAPOR TENSION.

The pressure, expressed in millimeters of mercury, exerted by water-vapor in a closed space.

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

CAPILLARITY

Is the same for all kinds of tubes which the liquid will wet.

Is inversely proportional to the diameter of the tube.

Becomes inappreciable in tubes of about 2 cm. (¾ inch) diameter.

For parallel plates and concentric cylinders is about ½ that of tubes.

Lessens with increase of temperature by about .002 (.2%) of its height for each degree C.

Water (pure) at 18° rises in a tube of 1 mm. diameter 29.3 mm. (conventionally 30 mm.).

Mercury at 18° is depressed in a tube of 1 mm. diameter approximately 9.2 mm. (conventionally 9 mm.).

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