Variations on a Theme -- PLANT GROWTH

By JAMES F. BONNER

THE STUDY of plant sciences at the Institute was initiated in 1930 by the first chairman of the Biology Division, Thomas Hunt Morgan. Professor Morgan was particularly interested in developing the study of the mechanisms by which organisms grow and develop, and for this reason he chose to initiate work in the plant sciences, not with botanical work in general as is done at many other institutions, but rather by confining the development to a few carefully chosen aspects of plant biology. Three principal approaches to the study were started: first, the study of plant heredity; second, the study of photosynthesis; and third, the study of plant growth.

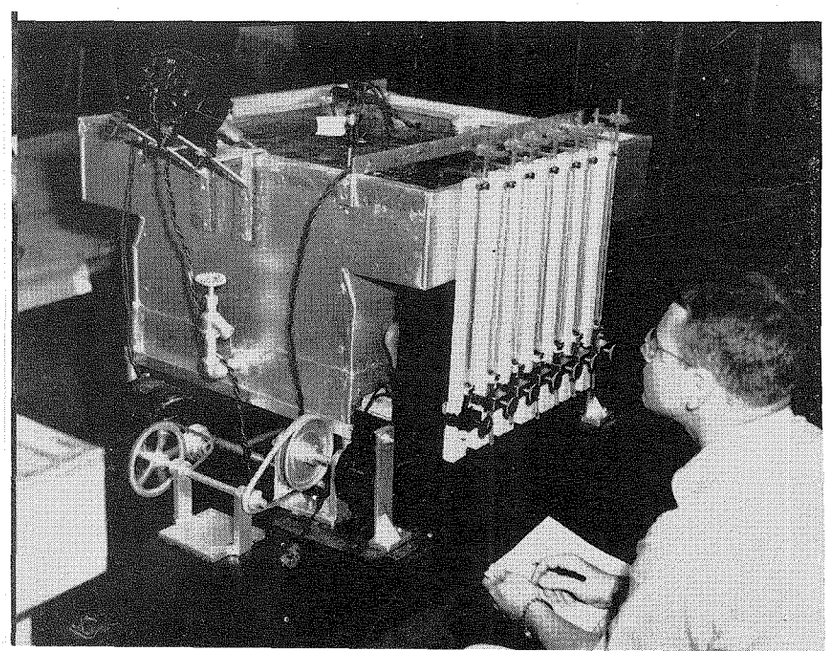
INDOLE ACETIC ACID

The work on plant growth was undertaken by Dr. Herman Dolk of the University of Utrecht, Holland, who became the first professor of plant physiology at the Institute. At that time it was known from work done in Holland by Dr. Dolk, F. W. Went (later a member of the Institute staff) and others that the growth of stems and stem-like organs of the plant is controlled by a particular chemical substance which is produced in the growing tip and which is transported through the stem to lower regions where it is essential for elongation. Dolk and K. V. Thimann, now professor of plant physiology at Harvard, undertook to isolate and identify the chemical nature of this growth promoting compound or growth substance. Their work, together with that af A. J. Haagen-Smit (now professor of bio-organic chemistry at the Institute) and others, established that the activity of the plant growth substance is possessed by a relatively simple organic substance, indole acetic acid. This and other related substances are produced in minute amounts in the tip of the growing plant, but are completely essential to growth of the stem, flower stalks, and other portions of the plant. In plants from which the growth substance producing tip has been severed, application of as little as 5 x 10^{-6} micrograms (5 x 10^{-12} grams) of indole acetic acid per plant suffices to bring about a clearly detectable increase in growth.

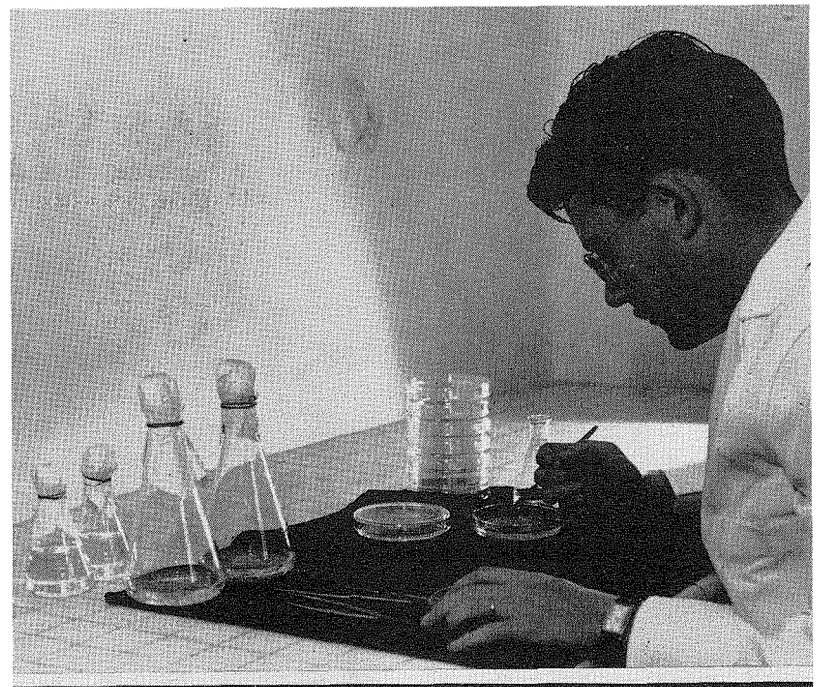
Within a short time it was discovered that the plant growth substance indole acetic acid regulates a wide variety of processes in the plant in addition to growth in length of stems, etc. Thus Thimann and Folke Skoog (now associate professor of Botany at the University of Wisconsin) found that the phenomenon of apical dominance by which the terminal or main bud of a shoot suppresses the growth of side buds is due to the same growth substance. In plants which had had their terminal buds removed, the side buds which normally grow out rapidly could be completely suppressed by application of indole acetic acid to the stump from which the main bud had been removed. The growth substance which is normally produced in the plant has then not only the property of regulating growth in size but also of regulating the branching or form of the plant. Professor F. W. Went, who joined the staff of the Institute in 1933 after the death of Dr. Dolk in an automobile accident in 1932, discovered a further and more important role of indole acetic acid in 1935 when he found that the application of this substance to cut stems of plants causes the formation of roots at the base of such material. This finding was translated into practical application by Went and W. C. Cooper (now with the U. S. Department of Agriculture) and treatment with indole acetic acid or related compounds has become the worldwide standard for the induction of roots on cuttings or slips of plants which are to be propagated in this way. Treatment with root forming chemicals is used by nursery men on a wide variety of ornamentals such as **Camellia, Chrysanthemum,** etc., and also on economic plants such as fruit trees. Dr. Cooper, now one of the U. S. authorities in this field of plant physiology, was called on during the war to treat cuttings of quinine, **Derris** and other strategic plant materials in connection with the greatly increased need for these products, and carried on work in Puerto Rico and in Peru as well as in the U. S.

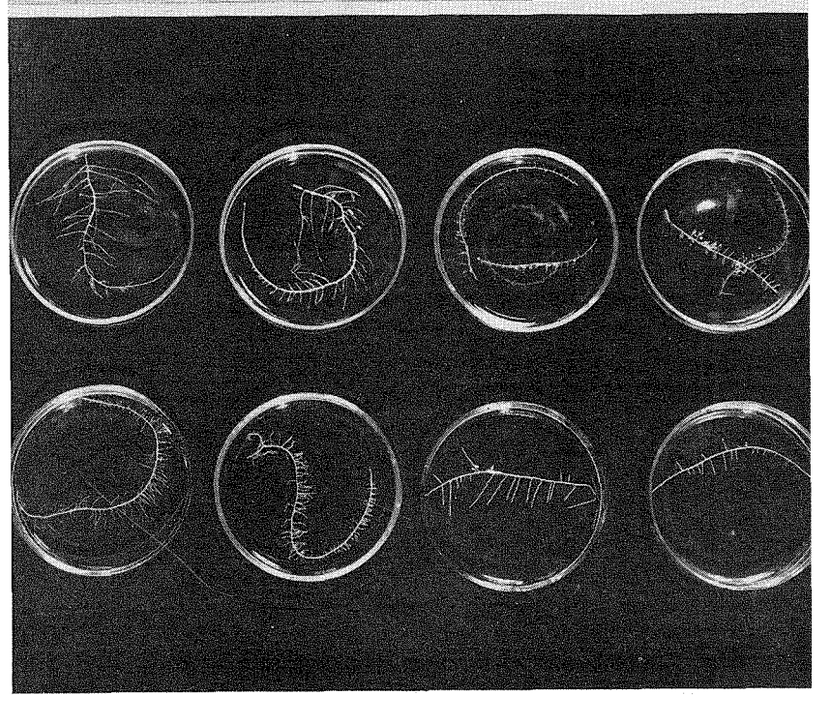
NAPHTHALENE ACETIC ACID AND 2-4-D

The study of the plant growth substance indole acetic acid initiated in this country at the Institute has been taken up by a great number of individuals and organizations, and steadily increasing numbers of applications in this and related compounds have been found in agriculture. Thus application of naphthalene acetic acid, a compound related to indole acetic acid and possessing growth substance activity, has been found to inhibit preharvest fruit drop, which has been a severe problem in certain apple growing areas. Application of the compound by sprays to apple orchards has now become an important agricultural practice. Similar sprays inhibit fruit drop of grapefruit and other citrus, as has been shown by William S. Stewart, a former student at the Institute,



Undergraduate John Thomas preparing to measure the rate of photo-synthesis of leaves in the Warburg Manometric apparatus.





UPPER: James Bonner culturing isolated plant roots. These roots are cut from the plants and are grown in nutrient solution for the purpose of finding which chemical substances are required in root growth. Here the roots are being cut into small fragments, each one of which will produce a new root. The flasks in the foreground contain flaxroots which have grown into pieces one meter or more long. The whole operation is carried out in an aseptic operating room. LOWER: Isolated roots grown in culture in nutrient solution. Upper left two dishes-tomato roots; upper right two dishes-alfalfa roots; lower left two dishes-clover roots; lower right two dishes-pea roots. All roots have grown for one week, and have increased 10-15 cm in length.

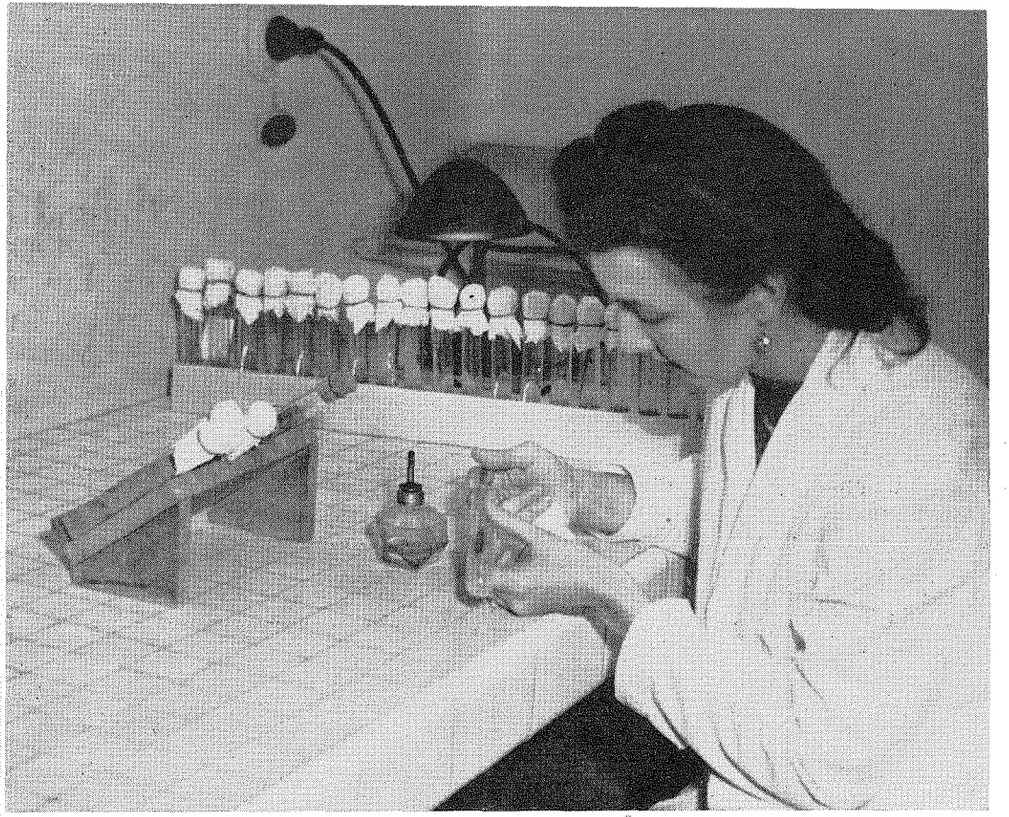
now assistant professor at the University of California. The compound 2-4 dichlorphenoxy acetic acid or 2-4-D, discovered as a product of work on plant growth substances, is highly toxic to some plants and is widely used as a weed killer. Dr. J. van Overbeek, formerly assistant professor at the Institute, now assistant director of the Institute for Tropical Agriculture at Mayaguez, Puerto Rico, has made an important application of this compound in combating weeds in sugar cane fields at a fraction of the cost required by hand weeding. Dr. Gladys King, now of the U. S. Department of Agriculture in New Orleans, has also made successful application of 2-4-D to the control of water hyacinth in the Mississippi River. The agricultural applications of this basic work carried on at the Institute are thus quite large and it should be clear that basic advances in agricultural technique can be expected as a result of work on the fundamental aspects of plant growth.

ROOT GROWTH SUBSTANCES

One of the phases of plant development which have been much investigated at the Institute is the growth of the root system. This has been studied particularly by Professor James Bonner and by F. T. Addicott, assistant professor at the University of California. The root normally grows at the expense of substances formed in the leaves which are transported to the roots and which there take part in the increase of the root system. This problem has been studied by the technique of isolated root culture, in which short pieces of aseptic root are transferred to aseptic nutrient solution and there allowed to grow. The root fragment will grow normally and produce a branched and vigorous root system, provided that the usual inorganic salts (which would ordinarily be taken from the soil), sugar (as a source of energy) and certain root growth substances are all present. These root growth substances, like the stem growth substance, are needed in minute amounts, concentrations of the order of 0.1 mg per liter of nutrient. The root growth substances differ fundamentally from the indole acetic acid of the stem, however, and have in fact been identified as vitamins of the B group. Thus, thiamin, the antineuritic vitamin, is required for the normal growth of all species of roots thus far investigated. This compound which is formed in the leaves of plants and translocated to the roots, here it is used in root growth, is also an essential component of the plant food eaten by the animal. The same is true of pyridoxin and niacin, two other vitamins, both formed in the leaf and essential for root growth but also essential dietary components for the animal. The root lives then at the expense of the leaves, both as to food supply (sugar) and as to essential root growth substances. Through its supplying of these materials to the root, the leaf can in turn regulate the growth of the root and can in this way adjust the root system to the demands of the aboveground portion of the plant. Practical applications of our knowledge of the root growth substances have been found in the promotion of root growth of plants or cuttings in particular instances.

LEAF GROWTH SUBSTANCES

Still another group of plant growth substances is represented by the factors which influence leaf growth. David Bonner, now assistant professor of Botany at Yale University, has studied leaf growth, using portions of excised immature leaves which were found to grow when placed in solutions containing sugar and extracts of leaves or seeds. The leaf or seed extracts contain a specific leaf growth promoting material which was identified as the compounds adenine



Research assistant Jean Campbell is carrying out a surgical operation on an excised plant embryo. The embryo is to be grown under aseptic conditions on antiseptic nutrient solution for the purpose of determining the nutritive requirement of the young plant.

and hypoxanthine. These substances are produced in mature leaves and appear to act in regulating leaf growth in much the same way that the stem and root growth substances regulate their respective organs.

WOUND HEALING AND PLANT TUMORS

Wound healing is an important process in plants as it is in animals, and it has long been known that when plant cells are cut or otherwise injured they liberate substances which tend to promote the proliferation of adjoining uninjured cells. The chemistry of plant wound healing was taken up at the Institute by J. Bonner and A. J. Haagen-Smit, and by James English, Jr., now associate professor of chemistry at Yale University. A powerful wound substance was isolated in pure form and found to be decene dicarboxylic acid, a compound new to organic chemistry. Practical applications of this interesting substance still remain to be made.

The study of the cancer of animals has its counterpart in certain tumor-like growths of plants and it appears from work of Skoog and others that the growth of these plant tumors is closely related to indole acetic acid. Plant tumors, which develop on stems under certain conditions, can grow in aseptic culture in the absence of any added growth substance and can in fact produce indole acetic acid. Nontumorous normal stem also can grow in culture but apparently only if supplied with indole acetic acid. Thus the tumorous condition might appear to be connected with the ability of the tumor tissue to form its own supply of this growth substance, so that it grows independently rather than dependently and harmoniously with normal tissues. This apparent relation of the tumorous condition in plants to plant growth substances is a field of active investigation at the present time.

A picture of plant growth as controlled and integrated by internal secretions of the plant has begun to emerge as a result of the work discussed briefly above. Each part of the plant depends for its growth on particular compounds which it cannot synthesize but which it obtains from other organs. In this way integration of the growth of the several organs appears to be brought about. It would be of great interest to know in similar detail the physiology of the formation of flowers and fruits, and obviously such knowledge, together with the implied ability to influence and regulate flowering and fruit production, would be potentially of agricultural significance. Only a small beginning in the study of these complicated problems, however, has been made and the study of the growth substances involved in flowering and fruiting remains a challenging problem to plant physiology.

MODE OF ACTION OF GROWTH SUBSTANCES

We have thus far taken up the growth of the plant from the standpoint of the relation of particular natural plant compounds to the expression of plant growth. Work actively under way at the Institute concerns the biochemistry of the growth substances, the way in which they carry out their striking growth effects. The growth substances thus far studied appear to enter into basic cellular reactions as components of enzyme systems. Thus thiamin, nicotinic acid, adenine, and pyridoxin all are known to be components of plant respiratory enzymes. The mode of action of indole acetic acid, on the contrary, has remained a mystery despite long continued study of the matter at the Institute and elsewhere. Recently enzyme systems, in which indole acetic acid participates in the plant, have been studied by James Bonner and S. G. Wildman. Enzyme systems for both the production and destruction of the growth substance have been separated and characterized. In addition a protein which appears to contain indole acetic acid as a constituent has been isolated from the plant and characterized as an enzyme. The study of the plant enzyme systems involved in growth may be expected to lead us to a new and deeper understanding of plant development and this biochemical phase of plant biology is being stressed at the Institute. Modern equipment and the best of facilities for plant enzyme and protein work are making it possible to make rapid progress in this new field.

GUAYULE

This discussion of Plant Physiology and Biochemistry at the Institute would be incomplete without some discussion of the work carried on by the Institute group and their past students during the war. The facilities of the plant physiology group were used during the war for the study of natural rubber production. This work was started by the Institute in 1940 and was continued during the war under contract with the Emergency Rubber Project of the U. S. Department of Agriculture. Work at the Institute involved determination of optimum conditions for production of rubber by rubber forming plants. Thus it was found with the aid of the air-conditioned greenhouses described elsewhere that in the guayule rubber accumulation takes place best under conditions of warm days and cold nights. The exact temperature data obtained permit ready selection of regions for guayule production which will have suitable climate for rubber accumulation. This work, together with basic investigations on the physiology of rubber formation, was carried out primarily by James Bonner, Arthur Galston, who will shortly return to the Institute after having been agricultural officer for the Naval Military Government Unit for Okinawa, and H. K. Pratt, now assistant professor of truck crops at the University of California (Davis). The oils and resins of rubber and rubber plant were investigated at the same time by Haagen-Smit and by Ralph Siu, now director of the Quartermaster Corps Biological Laboratory in Philadelphia. A basically new method for extracting rubber of highest quality from guayule was developed at the Institute by Professor Robert Emerson, now at the University of Illinois. As a result of the intensive work on natural rubber production carried on during the war, small but vitally needed quantities of natural rubber were produced for blending with synthetic rubber, and in addition, the guayule plant was established as a practicable source of domestic natural rubber.

Varied as are the above described research problems carried on in plant physiology and biochemistry, there are still others subjects which have been or are under investigation. Active investigations carried on at the present time by graduate students also include substances given off by plants and toxic to other plants, the biochemical explanation of genetically controlled dwarf structure, the mechanism of formation of organic acids in leaves, the production of carotenes, and others. This wide multiplicity of problems has its common ground in the fact that they apply to the varied aspects of plant growth. As has been shown above, the results of this type of study find their application not only in the better understanding of a general biological problem but also in rather direct ways to the bettering of agricultural procedures. That the training given in Plant Physiology and Biochemistry prepares the student not only for academic work but also for work in agriculture is attested by the success of the graduates of the Biology Division in the most varied phases of agricultural endeavor.

Research fellow Dr. Barbarin Arrengúin-Lozano studying cultures of isolated bark which he has made of the rubber plant Guayule. Dr. Arregúin-Lozano is using these bark cultures in the study of rubber formation.