Meetings

Plant Growth Regulation

Plant growth regulation has in the past 12 years witnessed two striking developments. On the one hand, auxin, which for about 30 years had been the only recognized major plant hormone, was joined by several new hormones: the gibberellins, the cytokinins, and, quite recently, abscisin-dormin and ethylene. (Both gibberellin and ethylene have much longer histories as regulators of plant growth, but their significance was not fully appreciated.) On the other hand, advances in molecular genetics made it possible to study growth and development in terms of gene activity and to investigate relations between gene and hormone action. These developments were reviewed at a seminar on Plant Growth Regulation, held 22–26 March 1966 in Kyoto, Japan. The seminar was part of the U.S.–Japan Science Exchange Program which is sponsored and supported by the National Science Foundation and the Japanese Society for Promotion of Science. Thirteen American and about 45 Japanese investigators attended.

The program consisted of two major parts, corresponding to the two major developments just mentioned. The first part treated advances in our knowledge of plant hormones as such—their structure, biochemistry, and physiology. K. V. Thimann (University of California, Santa Cruz) reviewed recent advances in the chemistry and physiology of auxins. With one apparent exception—the citrus auxin of L. N. Lewis—indoleacetic acid (IAA) and some of its derivatives remain the only compounds known to be naturally occurring auxins. Concentrations of IAA of 1 milligram per liter and above cause evolution of ethylene in segments of pea stems and other plant tissues (Burg and Burg). Thus, many effects hitherto ascribed to concentrations of auxin greater than those causing optimum growth are actually caused by ethylene. However, ethylene does not seem to be evolved by coleoptiles; hence this explanation does not seem to hold for this "classical" test material for auxins. Our knowledge of IAA biosynthesis does not seem to have progressed. The role of tryptophan as a precursor of IAA, for many years widely accepted, has come under doubt. Growth of sterile sections of *Avena* coleoptiles is not promoted by tryptophan, and tissue cultures do not convert ^{14}C-tryptophan to ^{14}C-IAA. In contrast, the destruction of IAA, at least of exogenous IAA, mediated by oxidases clearly seems to play a significant role in growth regulation. Naturally occurring phenols, widely distributed in plants, apparently modify IAA destruction and thus, participate in the regulation. Diphenols inhibit destruction of IAA and enhance growth; monophenols have the opposite effect. During transport of ^{14}C-IAA through tissue, a substantial part of the auxin becomes bound, but in the form of unchanged IAA, probably by hydrogen bonds to a protein.

P. M. Ray (University of California, Santa Cruz) has shown a clear-cut effect of auxin on metabolism in the cell wall. Tritium-labeled glucose was incorporated into the cell walls of coleoptile sections of *Avena*. The incorporation into the outer epidermal wall followed a distinct gradient, which was greatest at or near the inner surface of the wall, next to the protoplast. The gradient decreased to a relatively weak but definite level in the outer half, extending to the outermost edge. In the presence of auxin, incorporation in the inner portion of this wall (from approximately 0.15 to 0.65 of the distance from inner to outer edge) was substantially greater than that in controls. The results suggest that internal incorporation of material in the cell wall helps bring about the relaxation of stress in the wall which is necessary for cell growth, and that auxin affects this process by influencing the transport processes responsible for the internal incorporation.

T. Koimizu, Y. Isogai, and T. Okamoto (University of Tokyo) reported improved methods, based on gas chromatography and colorimetry, for identification and estimation of IAA, some IAA derivatives, and some phenolics in plant tissues. M. Konishi (Kyoto University) has investigated relations between the growth of a tissue (hypocotyls of the Japanese morning glory *Pharbitis nil*), as influenced by light, and its auxin content as well as other features of its metabolism. In this tissue the effect of light on growth is primarily related to an activation of carbohydrate metabolism rather than to changes in the hormone metabolism. Hypocotyls from seedlings treated with far-red light and exhibiting the highest growth rates were the only ones able to make growth in solutions containing up to 2 percent sucrose; all others were inhibited at 0.5 percent.

A. Lang (Michigan State/AEC Plant Research Laboratory, East Lansing) reviewed advances in the physiology of the second major group of plant hormones, the gibberellins. Gibberellins have been shown to promote a variety of phenomena of plant growth and development. The approach was, however, pharmacological (application of the regulator to plants or plant parts) and thus did not permit rigorous conclusions about the function of the hormone in the endogenous regulation of the individual responses. In the solution of this problem, some of the so-called growth retardants or dwarfing agents have proven a very useful tool. Several growth retardants, particularly 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride (AMO-1618) and 2-chloroethyltrimethylammonium chloride (CCC or Cycocel), are selective inhibitors of gibberellin biosynthesis in plants. The classical method in hormone physiology is removal of the hormone-producing organ by excision. In the case of the gibberellins, the hormone can be removed by "chemical surgery." By this technique it was possible to prove that gibberellins are indeed factors in the endogenous regulation of a multitude of specific plant growth processes, such as shoot elongation, flower initiation in certain plant types, sex expression (the determination of male and female flowers) in cucurbits, and other processes. Lang also reviewed briefly some results obtained with a new method for gibberellin determinations in plant organs, diffusion into agar, developed by Jones and Phillips. Extrac-

tion of the tips of pea seedlings yields two gibberellins, most probably GA_1 and GA_5. However, diffusion yielded only one, the GA_1. GA_5 thus seems to be present in a non-mobile, perhaps loosely bound form in the cells.

Interconversions and "binding" of gibberellins were also reported by other speakers. B. O. Phinney and coworkers (University of California, Los Angeles) showed that gibberellins A_4 and A_7 are precursors of A_1 and A_3, respectively, at least in the fungus *Gibberella fujikuroi* (*Fusarium moniliforme*). This conversion is under the control of one gene; total gibberellin production is under the control of another. It has been known for some time that immature seeds of many plants are strikingly rich in gibberellins, but that this level undergoes a substantial decline with maturation of the seeds. L. Rappaport (University of California, Davis) demonstrated the presence of inactive, "bound" gibberellins in bean seeds and also found some evidence for binding of C^{14}-gibberellic acid by mature seeds. These results indicate that the active gibberellins are converted into inactive or bound ones as the seeds mature.

The number of chemically identified gibberellins at the time of the seminar was 13. The Japanese, who discovered the first gibberellins, are very active in the search for further members of this class of hormones and for related compounds. S. Tamura, N. Takahashi, N. Murofushi (University of Tokyo), and J. Kato (Osaka Prefecture University) reported on the isolation and identification of a new gibberellin from bamboo shoots. The gibberellin belongs to the C_{19} series but has two carboxyl groups and no lactone in the A ring. T. Hashimoto (University of Tokyo) undertook a search for anti-gibberellins which would be of obvious and great use in studies on gibberellin action at the molecular level. Three compounds, deoxygibberellin C and the methyl esters of deoxygibberellin C and of epigibberellin C, were found to have certain anti-gibberellin properties. The mode of action requires, however, further studies. A complicating finding, although very interesting in itself, is that the effect of these compounds varies with the gibberellin with which they are combined. Thus, deoxygibberellin C inhibited the growth-promoting effect of gibberellin A_9 and of the bamboo gibberellin on seedlings of d_5-dwarf maize, however the deoxygibberellin enhanced the effects of several other gibberellins. Interactions of applied gibberellin and auxins on flowering in chrysanthemums and fruiting in tomatoes were reported by Y. Tsukamoto and T. Asahira (Kyoto University).

The topic "cytokinins" was introduced by F. Skoog (University of Wisconsin, Madison). Comparative tests of approximately 70 purine derivatives for growth promotion in cultures of tobacco tissue lead to the conclusion that only 6-substituted purines possess cytokinin activity and further that an unsubstituted 1-position is a requirement for such an activity. C. O. Miller (Indiana University, Bloomington) presented evidence that zeatin, the only identified cytokinin from higher plants, occurs in young maize kernels not only in the free form, but also in the form of a nucleoside and a mono- and possibly also a diphosphate nucleotide. During development of the kernel, the free base or the riboside appear first and the ribotide builds up later. K. Koshimizu and S. Matsubara (Kyoto University and Kyoto Prefectural University) reported on the partial identification of a cytokinin-like factor from immature lupin seeds. It is a purine derivative and seems identical with or closely related to zeatin.

A number of very interesting results of cytokinin studies at the University of Tokyo and Tokyo University of Agriculture and Technology were summarized by S. Kuraishi (of the former institution). 4-Benzylaminobenzimidazole was very effective in maintaining chlorophyll in detached, senescing leaves. Although the test is different from that used by Skoog it seems possible that certain substituted benzimidazoles also possess cytokinin activity. Studies on the effect of kinetin on incorporation and release of C^{14}-leucine in leaf protein indicated that the main effect of the regulator is reduction of protein degradation rather than enhancement of protein synthesis. Finally, it was found that treatment with benzylaminopurine, a synthetic cytokinin, greatly increased the cold tolerance of all plant species so tested; the resistance to herbicides and pesticides was also increased. Since cytokinins are also known to increase plant resistance to other stress conditions they appear to be veritable phytoaspirins.

F. T. Addicott (University of California, Davis), the discoverer of abscisin II, gave a thorough survey of the known effects of this latest member of the plant hormone family. Abscisin seems to be mainly involved in situations where the growth activity of a plant or plant organ is to be reduced or terminated. This hormone is important in induction and maintenance of dormancy conditions, in the abscision (shedding) of aging organs, and generally in the onset of senescence. Abscisin appears to be a phytotranquillizer.

The second part of the seminar dealt with interrelations between nucleic acid and protein synthesis in the growing cell, and the effect of plant hormones on the growth of this cell. H. Stern (University of California, San Diego) reviewed new results on regulation of DNA synthesis found in studies on the experimental control of meiosis. Meiotic cells show a second period of DNA synthesis in mid-prophase which is critical to completion of the process. Meiosis seems to result because of a delay in this synthesis; pre-meiotic cells can be prevented from entering mitosis and caused to enter the meiotic cycle by treatment with a DNA inhibitor at the appropriate time. These results are of great general significance. They indicate that the general view of DNA synthesis being restricted to the "S" phase of the "cell cycle" may be an oversimplification. There are aspects of DNA synthesis which do not primarily reflect gene reproduction but have an important bearing on chromosome behavior and the regulation of mitosis and meiosis.

F. C. Steward (Cornell University, Ithaca) discussed relations of protein synthesis and turnover to the induction of growth in plant cells. Plant cells cultured on a basal medium may increase considerably (15 to 20 times) their nucleic acid content, but do not increase their protein content. Addition of factors which stimulate cell division (coconut-milk factors) stimulate protein synthesis and turnover. These effects, as shown by electron microscopy, extend to virtually all cell organelles. These factors may thus be specifically needed for making RNA effective in protein synthesis and growth. Another important aspect is the existence of distinct pools or "phases" of amino acids. Different pools of a given acid do not mingle in the cell and may be available for protein synthesis and growth to a very different extent. Thus, glutamic acid en route to protein may receive its carbon and sometimes also its nitrogen from the nutrient medium, while the main endogenous pools of soluble compounds remain largely untouched. Access of cells to exogenous compounds may thus be an important

factor in their growth regulation, despite presence of the same compounds in the cells themselves. K. Syono (Kitasato University) demonstrated marked differences in the metabolism of nucleic acids of plant tissues which had been cultured for different lengths of time. These differences were apparent both in the ability for incorporation of certain precursors (C^{14}-guanine), and the ability for response to such precursors or to kinetin.

Relations between plant hormone action on the one hand, nucleic acid and protein synthesis on the other was the single topic of the seminar which received the greatest attention. J. L. Key (Purdue University, Lafayette) reviewed his work showing close correlation between cell growth and synthesis of a particular, DNA-like RNA fraction. The fraction is most probably a messenger RNA, which in turn is presumably associated with the synthesis of proteins (enzymes) serving a function in growth. There is as yet no direct demonstration of an auxin-enhanced synthesis of this or any other specific RNA in the cells. But it is clear that auxin can promote cell growth only to the extent that the tissue is capable of synthesizing RNA (presumably messenger) and protein; there is indirect evidence that the RNA and protein essential for cell growth are increased by auxin. T. Yamaki (University of Tokyo) developed farther reaching and more specific ideas about the relations among auxin and RNA and protein synthesis. His theory is based particularly on the finding that in short-term feeding experiments with C^{14}-IAA the auxin appears predominantly associated with a microsome-rich tissue fraction. Two very interesting findings were reported by S. Wada (Kobe University) and Y. Masuda (Osaka City University): (i) Treatment of tissue with actinomycin D inhibits not only auxin-induced growth but also, contrary to results of R. E. Cleland, auxin-induced stress relaxation in the cell wall; and (ii) auxin causes increased RNA synthesis (C^{14}-uracil incorporation) in the treated tissue even if the latter is inhibited from active growth by means of an osmotic stress (incubation in mannitol solution).

These findings support the possibility that the effect of auxin on RNA synthesis is a direct and primary effect of the hormone. N. Yanagashima and Masuda reported that the tissue of the Jerusalem artichoke tuber, which responds to auxin only after a lag period, becomes responsive to auxin after a treatment with gibberellin. From tissue so treated a fraction could be isolated, by all available criteria an RNA, which had a quite similar effect on the tissue. From these results it was concluded that the action of auxin in the tissue is dependent on a specific condition, apparently presence of a specific RNA. The RNA in tissues responsive to auxin is generated endogenously, while in others it can be induced under the influence of gibberellin. Similar effects were also found in yeast, thus raising the possibility that yeast cells have a mechanism of cell growth in common with higher plants. The effects were however relatively small (usually not over 15 percent); some questions were raised as to their specificity. J. E. Varner (MSU/AEC Plant Research Laboratory) summarized his studies on hormonal control of enzyme synthesis in the aleurone layer of barley endosperm. Gibberellin in this tissue causes increases in the activities of α-amylase, protease, ribonuclease, and other hydrolytic enzymes. At least for α-amylase it could be conclusively shown, by means of a new method using density-labeling of "new" protein with a heavy isotope, that the total increase is based on *de novo* synthesis; it may be assumed that this in turn is dependent on RNA synthesis.

Several papers, while not dealing with relations between plant hormones and RNA and protein synthesis, provided interesting extensions or additions to the barley endosperm work. H. Yomo and H. Iinuma (Takara Shuzo Company, Ltd., Kyoto) showed that the embryo of the dry barley seed does not contain measurable quantities of gibberellin, neither in free nor in bound form, but that gibberellin synthesis is initiated a short time after imbibition. The amounts of hormone synthesized are such as to half-saturate the production of amylase in the aleurone, thus, very likely, ensuring a continuous and adequate flow of nutrients back to the embryo. It is not understood whether gibberellin exerts its control of enzyme synthesis in the aleurone at the transcription or the translation level (or perhaps at either). It remains to be seen what factors are essential in the control of gibberellin synthesis by the embryo. Nevertheless, even as it stands now the barley "system" is one of the best understood cases of growth regulation both at the organ and the molecular levels. It is in particular that case where *de novo* synthesis of a particular enzyme under the action of a hormone has been most clearly demonstrated.

Y. Murakami (National Institute of Agricultural Sciences, Tokyo) and S. Imamura and Y. Ogawa (Kyoto University) have used amylase production by rice endosperm as a test for the determination of gibberellin-like substances. Rice endosperm is superior to barley endosperm insofar as the basal amylase level is almost zero. The method permitted recognition of active substances in a wide variety of organisms, including animals (silkworm). Some of these substances were not active in gibberellin assays based on growth promotion. In both plants and animals, very definite changes of gibberellin content were found during development.

The most vigorous of the general discussions dealt again with the relations among plant hormones and nucleic acid and protein metabolism. J. Bonner (California Institute of Technology, Pasadena) reviewed advances in this area. He proposed the thesis that plant growth hormones function, partially or generally, as effector substances for RNA production. Lang pointed out, in contrast, that with the exception of the barley aleurone system none of the reported findings on RNA or protein increases after hormone treatment provided conclusive proof that the latter was the direct cause of the former. All that these findings show is that nucleic acid and protein synthesis have to proceed at a certain rate if the hormone is to exert its full effect on cell growth. It is also entirely possible to envisage hormone regulation at levels other than the "genetic" one, for instance at the level of the completed protein molecule (enzyme activation), or that of cell membranes which may control the accessability of enzyme, substrate, and so forth. Other speakers pointed out problems of timing. Effects of added auxin on growth of a tissue can be measured within 10 to 15 minutes and perhaps even faster (Ray); effects on cytoplasmic streaming are visible in as little as 2 minutes. The half-life of Key's DNA-like RNA, which is so closely correlated with growth, is on the other hand about 2 hours; the shortest half-life of an RNA known in higher organisms is 7 minutes, and in most cases the half-life seems to be considerably longer. If half-life is a measure for rapidity of synthesis, then no RNA is known in higher organisms which would explain the fastest responses to

plant growth hormones which have been measured. Despite such questions, however, the consensus was that Bonner's hypothesis was so clear, and so much within the reach of available techniques, that—as Varner put it—we have no choice but to test it.

The meeting provided an excellent opportunity for a thorough comparison and evaluation of new findings in the plant hormone field. It was unanimously decided to make an effort to organize another, similar meeting after 3 or 4 years.

ANTON LANG
MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing

Calendar of Events

National Meetings

August

13–16. **Soil Conservation** Soc. of America, 22nd annual meeting, Des Moines, Iowa. (7515 NE Ankeny Rd., Ankeny, Iowa 50021)

13–17. **Energy Conversion** Engineering, 2nd conf., Miami Beach, Fla. (ASME, 345 E. 47 St., New York 10017)

14–16. **Astrodynamics,** Guidance and Control, conf., Huntsville, Ala. (American Inst. of Aeronautics and Astronautics, 1290 Sixth Ave., New York 10019)

14–17. National **Medical** Assoc., St. Louis, Mo. (S. C. Smith, 520 W St., NW, Washington, D.C. 20001)

15–18. American **Dietetic** Assoc., Chicago, Ill. (R. M. Yakel, ADA, 620 N. Michigan Ave., Chicago)

20–23. American **Phytopathological** Soc., Washington, D.C. (J. P. Fulton, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville 72701)

20–25. American **Crystallographic** Assoc., Minneapolis, Minn. (T. Zoltai, Dept. of Geology and Geophysics, Univ. of Minnesota, Minneapolis 55455)

20–25. American Soc. of **Parasitologists**, 42nd annual mtg., Tucson, Ariz. (D. V. Moore, Univ. of Texas, Southwest Medical School, Dallas 75235)

21–23. **Cryogenic Engineering**, conf., Palo Alto, Calif. (K. D. Timmerhaus, c/o Engineering Center, AD 1-25, Univ. of Colorado, Boulder)

21–25. American Assoc. of **Clinical Chemists**, 18th annual mtg., Philadelphia, Pa. (M. E. Rylan, 318 Rodman Ave., Jenkintown, Pa. 19046)

21–25. **Complex Problem Solving**, conf., Andover, N.H. (S. S. Cole, Engineering Foundation, 345 E. 47 St., New York 10017)

21–25. **Poultry Science** Assoc., 56th annual mtg., Durham, N.H. (C. B. Ryan, c/o Texas A&M Univ., College Station 77843)

23–25. **Estuarine Pollution,** natl. symp., Stanford, Calif. (R. M. Kennedy, Kennedy Engineers, 604 Mission St., San Francisco, Calif. 94105)

23–25. **Gas Dynamics** Symp., Evanston, Ill. (The Symposium, Northwestern Univ., Evanston)

23–25. Wave Propagation and Dynamic Properties of **Earth Materials,** symp., Albuquerque, N.M. (G. E. Triandafilidis, Univ. of New Mexico, P.O. Box 188, University Station, Albuquerque 87106)

23–26. American **Physiological** Soc., fall mtg., Washington, D.C. (Executive Secretary, 9650 Wisconsin Ave., Bethesda, Md.)

24–26. **Phytochemical** Soc. of America, annual mtg., Madison, Wis. (T. J. Mabry, Univ. of Texas, Austin 78712)

27. American Assoc. of **Electromyography and Electrodiagnosis,** annual mtg., Miami Beach, Florida. (M. K. Newman, 16861 Wyoming Ave., Detroit, Mich. 48221)

27–31. American Soc. for **Pharmacology and Experimental Therapeutics**, fall mtg., Washington, D.C. (Executive Officer, 9650 Wisconsin Ave., Bethesda, Md.)

27–1. American Congr. of **Physical Medicine and Rehabilitation**, 45th annual session, Miami Beach, Fla. (Executive Director, 30 N. Michigan Ave., Chicago, Ill.)

27–1. American Inst. of **Biological Sciences**, 18th annual mtg., College Station, Tex. (AIBS, 3900 Wisconsin Ave., NW, Washington, D.C.)

The following societies will meet in conjunction with the AIBS. Additional information is available from AIBS or from the program chairmen listed below.

American **Bryological** Soc. (Secretary Treasurer, Box 36, S.W. Missouri State College, Springfield)

American Soc. for **Horticultural Science**. (Executive Director, 615 Elm St., St. Joseph, Mich. 49085)

American Soc. of **Human Genetics**. (c/o Division of Medical Genetics, Dept. of Medicine, Johns Hopkins Hospital, Baltimore 5, Md.)

American Soc. of **Naturalists**. (Executive Director, 3900 Wisconsin Ave., NW, Washington, D.C. 20016)

American Soc. of **Plant Physiologists**. (Secretary, c/o Dept. of Biology, Yale Univ., New Haven, Conn.)

American Soc. of **Plant Taxonomists**. (Secretary, c/o Botany Dept., Univ. of California, Berkeley)

Botanical Soc. of America. (Secretary, c/o Botany Dept., Indiana Univ., Bloomington)

Ecological Soc. of America. (Secretary, c/o Ecology Section, Health Physics Div., Oak Ridge National Lab., Oak Ridge, Tenn.)

Genetics Soc. of America. (Executive Director, 3900 Wisconsin Ave., NW, Washington, D.C. 20016)

Mycological Soc. of America. (Secretary-Treasurer, c/o Pioneering Res. Div., Natick Labs., Natick, Mass.)

28–30. Gatlinburg Conf. on Special Topics in **Nuclear Education and Research**, Gatlinburg, Tenn. (J. E. Mott, Oak Ridge Associated Universities, Box 117, Oak Ridge, Tenn. 37830)

28–30. Preparation and Properties of **Electronic Materials**, 9th annual conf., New York, N.Y. (L. R. Weisberg, RCA Labs., David Sarnoff Research Center, Princeton, N.J. 08540)