JOHN JEYES LECTURE*

Chemicals which Control Plant Growth

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1 Introduction

It is indeed an honour for me to have been chosen to receive the first John Jeyes Medal and Lecture Award. In doing so I wish to pay tribute to an outstanding man. His lively research interests and business acumen led him to important achievements whose impact on society are still very evident today.

John Jeyes, born in 1817, was the second son of Northampton parents. He developed interests in botany and horticulture as a boy and these interests stayed with him throughout his life. His name has been associated with the breeding of the 'Jeyes Conquerer' pea—though some would have it that the seed actually came from an Egyptian tomb! At any rate, this quite famous variety was developed by John Jeyes. When he came to London in 1859 he found that conditions amongst the poor were appalling: hygiene was non-existent and disease was prevalent everywhere. Aware of Lister's classical work on phenol, Jeyes began to study coal tar and the tar acids in particular. In 1877, one hundred years ago, he took out his first British patent on disinfectants. Not long afterwards he set up a company, 'Jeyes Sanitary Compounds Co. Ltd.', to handle what was to become the famous Jeyes Fluid.

So John Jeyes was a pioneer in the development of chemicals with biological activity and he was also interested in plants. Perhaps this is the reason why the Council of the Chemical Society decided that this, the first John Jeyes Lecture, should be concerned with chemistry and with plants.

Now plants, directly or indirectly, provide all the food of man and animals so a knowledge of how they grow is of first-rate importance.

Let us consider what happens when a seed germinates. It sends down a root to collect water and nutrients from the soil and produces a shoot which when it appears above the ground can take in carbon dioxide from the air and absorb energy from the sun. Thus provided with energy and an adequate supply of food and water, it has the wherewithal to grow. By a process of cell division new cells are produced in the tips of shoot and root and when these tiny cells enlarge—mainly by taking in water—the tissues increase in size and growth results. Then there must also be something in seeds which will ensure that the plants which grow from them will be true to type, with the same form, size, and structure as

*Based on the John Jeyes lecture first given at the Chemical Society Annual Congress in London on 31st March 1977.

all other plants of the same kind. These controlling influences in the seed we call genetic. They are dependent on hereditary factors which, carried in the nucleus of cells, come down to the plant from its parents. Quite a lot is known about plant growth in relation to its nutrition and more and more is being discovered about the chemistry of its genetic make-up, but there is another aspect of plant growth which is no less important. Why should all the various growth processes occur just when and how they do? Why should plants always bend towards the light? Why should roots appear at the base of a cutting and why should a ripe apple fall from the tree? We now know that such processes are controlled by a complex of growth hormones and inhibitors, all of which are extremely potent chemicals produced by the plant itself.

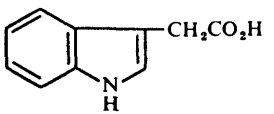
No account of this fascinating chapter of botanical research would be complete without reference to Charles Darwin who in 1880, presenting his findings in a book 'The Power of Movements in Plants', came to the conclusion that some 'influence' was operating from the tip of a shoot which made the plant respond to light.[1] This 'influence' of Darwin's is now known to be exerted by growth hormones which, synthesized in the growing tip of a shoot, diffuse downwards and promote growth by causing the cells to enlarge. Two types of cell elongation hormone are now recognized—the *auxins* and the *gibberellins*. The other fundamental process which is concerned in plant growth is cell division by which new cells are produced. Naturally occurring compounds which can influence this process are called *cytokinins*. In addition, the gaseous hydrocarbon *ethylene* and two endogenous hormone inhibitors *abscisic acid* and *xanthoxin* operate in the complex of compounds which control the growth and development of plants.

Hormones and hormone inhibitors are also important in determining the plant's response to environmental factors such as light, temperature, and various stress conditions such as drought and waterlogging.

These then, are the chemicals which control plant growth about which I shall be talking in this address—with a 'fluidity' which I hope, would have met with the approval of John Jeyes himself!

2 The Auxins

Went in 1926 demonstrated that oat seedlings contain a diffusable substance which would promote their growth; this was the first clear indication that a growth hormone occurs in plants.[2] The discovery eight years later by Kögl[3] that indole-3-acetic acid (IAA) (1) is capable of promoting the elongation of plant cells



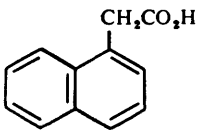
(1) Indole-3-acetic acid

[1] C. Darwin, 'The Power of Movement in Plants', John Murray, 1880.
[2] F. W. Went, *Proc. kon. ned. akad. Wetenschap.*, 1926, **30**, 10.
[3] F. Kögl, A. J. Haagen-Smit, and H. Erxleben, *Z. physiol. Chem.*, 1934, **228**, 90.

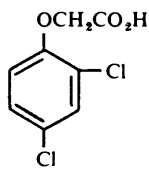
focused attention on this compound which is now recognized to be the most important of the class of growth hormones known as the *auxins*.

It is worth recording that IAA had been found in human urine over 50 years before[4] though it was not synthesized[5] until 1904. IAA is widely distributed in plants where it appears to be synthesized from tryptophan by a series of enzyme-controlled reactions.[6] The amounts of endogenous IAA are extremely small and are controlled at the physiological levels required for normal growth by the capacity of the plant to biosynthesize the compound, to destroy it by the action of an IAA oxidase,[7] and to conjugate the molecule with such compounds as amino-acids,[8] thereby removing its activity.

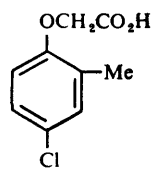
The discovery of IAA as an auxin led chemists to examine the growth-regulating activity of compounds with similar structures. As a result, a wide range of active compounds has become available. These include arylacetic acids [*e.g.* α-naphthylacetic (2) and 2,3,6-trichlorophenylacetic acids], aryloxy-acids [*e.g.* 2,4-dichlorophenoxyacetic acid (2,4-D) (3) and 2-methyl-4-chlorophen-oxyacetic acid (MCPA) (4)], and certain benzoic acids [*e.g.* 2,3,6-trichlorobenzoic acid (5)]. Certain 2,6-disubstituted phenols [*e.g.* (6)] are also active.[9]



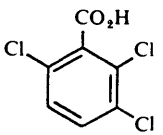
(2) α-Naphthylacetic acid



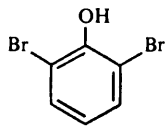
(3) 2,4-D



(4) MCPA



(5) 2,3,6-Trichlorobenzoic acid



(6) 2,6-Dibromophenol

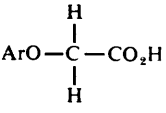
Unlike the natural auxin IAA, these synthetic growth regulators are not readily inactivated within plant tissues and some of them are therefore much more physiologically active than IAA itself. Commercial uses for these synthetic growth substances were soon found and include promoting the rooting of cuttings,

[4] J. P. Neaki and N. Sieber, *J. prakt. Chem.*, 1882, **26**, 333.
[5] A. Ellinger, *Ber. deut. Chem. Gesellschaft*, 1904, **37**, 1801.
[6] F. Wightman, *Canad. J. Bot.*, 1962, **40**, 689.
[7] Y. W. Tang and J. Bonner, *J. Arch. Biochem.*, 1947, **13**, 11.
[8] W. Andrea and N. E. Good, *Plant Physiol.*, 1955, **30**, 380.
[9] R. L. Wain and H. F. Taylor, *Nature*, 1965, **207**, 167.

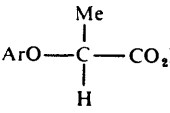
(Plate 1), reducing fruit fall, setting fruit in the absence of pollination (Plate 2), and selective weed control (Plates 1—7 are between pp. 274 and 275).

Much research has been carried out on the relationships between chemical structure and capacity to induce growth effects in plants. In the phenoxyacetic acids chlorine substituted in the ring affects activity. Thus, the low activity of phenoxyacetic acid increases in the order 2 > 3 > 4 in the monochloro-derivatives. In the dichlorophenoxyacetic acids, the 2,3-, 2,4-, and 3,4-isomers are all active but the 2,6- and 3,5-derivatives are not.[10]

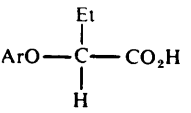
Studies on the effect of introducing alkyl groupings into the side-chain of aryloxyacetic acids (7)—(10) have shown that the molecule must have at least one hydrogen atom on the carbon adjacent to the carboxy-group for activity to be shown.[11,12]



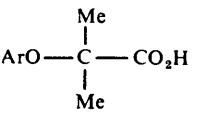
(7) Acetic derivative (active)



(8) Propionic derivative (active)



(9) Butyric derivative (active)



(10) Isobutyric derivative (inactive)

When the molecule possesses an asymmetric carbon atom, as with the above propionic and butyric derivatives, high activity is shown by one enantiomorph and the other is inactive.[13] It is of interest here to note that steric considerations also apply with certain cinnamic acids where the *cis*-isomer is extremely active as a growth substance and the *trans*-isomer is inactive.[14]

The literature on the mode of action of growth substances and on chemical structure in relation to plant growth-regulating activity is extensive and has been reviewed.[15,16]

A. Effects of Auxins on Enzyme Activities.—It would seem logical to expect that physiologically active compounds which are capable of controlling plant growth and development must exert influences on enzyme systems within the plant.

The effect of auxins on enzyme activity in inulin-containing plant tubers has recently been investigated in our laboratory.[17] Discs cut from the roots of chicory and Jerusalem artichoke were held in the surface of auxin solutions for varying

[10] R. L. Wain and F. Wightman, *Ann. Appl. Biol.*, 1963, **40**, 244.
[11] D. J. Osborne and R. L. Wain, *Science*, 1951, **114**, 92.
[12] C. H. Fawcett, R. L. Wain, and F. Wightman, *Ann. Appl. Biol.*, 1955, **43**, 342.
[13] M. S. Smith and R. L. Wain, *Proc. Roy. Soc.*, 1951, **B139**, 118.
[14] E. N. Ugochukwu and R. L. Wain, *Ann. Appl. Biol.*, 1968, **61**, 121.
[15] R. L. Wain and C. H. Fawcett, 'Plant Physiology', Vol. VA, Academic Press, New York, 1969.
[16] J. L. Garraway and R. L. Wain, in 'Drug Design', Vol. VII, Academic Press, New York, 1976.
[17] R. L. Wain, P. P. Rutherford, E. W. Watson, and C. M. Griffiths, *Nature*, 1965, **203**, 504.

periods at 25 °C. Considerable increases in the size and weight of the discs occurred (see Table). The effect was shown by a wide range of substances which

Table *Water uptake and invertase activities of chicory and Jerusalem artichoke discs after* 3 *days' treatment at* 25 °C

Tissue	*Treatment*	*Water uptake, increase as % of initial wt.*	*Units* of invertase activity* $\times 10^6$
Chicory	Fresh tissue	—	46.3
	Water	35.2	52.5
	10^{-5}M 2,4-D	280	3392
Jerusalem artichoke	Fresh tissue	—	0
	Water	24	0
	10^{-5}M 2,4-D	96	324

* 1 Unit represents 2 μmol hexose liberated per min at 25 °C per mg initial dry weight.

were active in standard tests for plant growth-regulating activity. Furthermore, structural requirements which have been established for auxin activity were found to operate in the water uptake response.[18]

In further studies it was shown that treatment of discs of Jerusalem artichoke with 2,4-D led to a breakdown of fructosans and the production of soluble reducing sugars within the tissues and also to an increase in the rate of respiration. It would therefore seem that the resulting changes of osmotic pressure and the provision of energy arising from the liberated sugars together promote the observed water uptake.

Since the changes in carbohydrate status within the tissues are initiated by the growth-substance treatment, studies have been made on the enzyme systems which might be involved.[19] Hydrolytic enzymes were extracted from discs of chicory and Jerusalem artichoke which had been treated with 2,4-D and also from the controls, and the extracts were fractionated on cellulose columns. The invertase activity is shown in the Table.

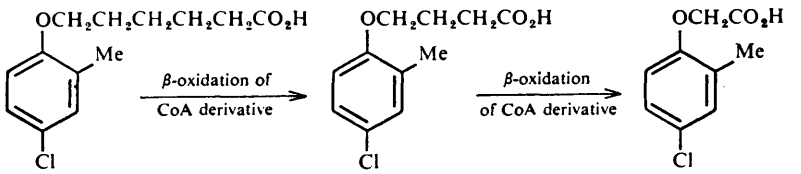
The increases in invertase activity observed in both tissues following treatment with 2,4-D could not be associated with an increase in enzyme protein, but this finding does not rule out the possibility that a small proportion of new enzyme with very high specific activity is produced. It is also possible that the growth-substance treatment caused the enzymes to become released from a bound form on the cell wall; alternatively, the activity of enzymes already present could have become greatly increased by the action of the growth substance.

B. Selective Weed Control.—Synthetic auxins such as 2,4-D and MCPA, when applied at low rates per acre to certain plant species, produce such drastic growth effects that the plants outgrow themselves and cannot survive; other species,

[18] P. P. Rutherford, C. M. Griffiths, and R. L. Wain, *Ann. Appl. Biol.*, 1966, **58**, 467.
[19] A. E. Flood, P. P. Rutherford, and E. W. Watson, *Nature*, 1967, **212**, 1049.

however, including cereals and grasses, are able to withstand these low doses and remain unharmed. Used in this way these chemicals are applied annually to millions of acres of crops to remove weed competition and they have made a tremendous contribution to world food production. Even so, their range of use is limited; for example, they are of no value for controlling weeds in clover, lucerne, and other legumes because these crops are just as susceptible to them as the weeds.

Some years ago, our basic studies on the oxidation of ω-substituted fatty acids within plant tissues led to a new type of herbicidal selectivity. The principle involved is logical; the susceptible plant when treated with a compound which is inactive *per se* converts it enzymically into a herbicide and this 'lethal synthesis' operating within the cells leads to destruction of the plant. An example of how selectivity can be achieved by this approach is provided by certain γ-phenoxybutyric and ϵ-phenoxycaproic acids.[20,21] When applied to susceptible species these compounds become converted into their coenzyme A derivatives which in presence of an appropriate β-oxidase enzyme system undergo β-oxidation of the side-chain (Scheme 1). In this way the highly herbicidal acetic acid derivative is produced, a molecule of acetylcoenzyme A being lost at each stage.



ϵ-(2-Methyl-4-chlorophenoxy)-caproic acid

γ-(2-Methyl-4-chlorophenoxy)-butyric acid (MCPB)

2-Methyl-4-chlorophenoxy-acetic acid (MCPA) (herbicidal)

Scheme 1

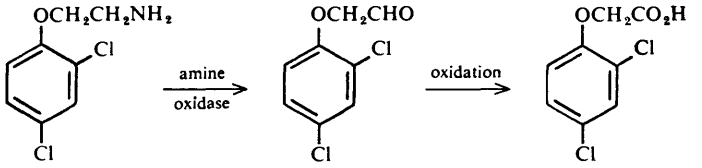
Certain legume crops possess a β-oxidase enzyme system which is not adapted to operate with these synthetic substrates. Degradation therefore does not occur and the crop plants remain unharmed although many weed species are destroyed.

Some 20 homologous series of phenoxy-acids were used in the physiological and biochemical investigations which established that the herbicidal selectivity of these butyric and caproic acids depends upon differential lethal synthesis.[22–24] The two compounds chosen for commercial development were γ-(2,4-dichlorophenoxy)butyric acid (2,4-DB) and γ-(2-methyl-4-chlorophenoxy)butyric acid

[20] R. L. Wain, *Ann. Appl. Biol.*, 1955, **42**, 151.
[21] R. L. Wain, *J. Agric. Food Chem.*, 1955, **3**, 128.
[22] R. L. Wain and F. Wightman, *Proc. Roy. Soc.*, 1954, **B142**, 525.
[23] C. H. Fawcett, R. M. Pascal, M. B. Pybus, H. F. Taylor, R. L. Wain, and F. Wightman, *Proc. Roy. Soc.*, 1959, **B150**, 95.
[24] C. H. Fawcett, R. L. Wain, and F. Wightman, *Proc. Roy. Soc.*, 1960, **B152**, 231.

(MCPB). Both of them are widely used in agriculture for selective weed control in certain legume crops and in cereals undersown with clover.[25,26]

Another example of lethal synthesis which has formed part of our research[27] is the conversion of aryl- and aryloxy-ethylamines into the corresponding aldehydes. This only occurs when the appropriate amine oxidase systems are present within the treated plant. The aldehyde so formed then becomes oxidized to the corresponding acid (Scheme 2) which, as with 2,4-D, may be strongly herbicidal.



2-(2,4-Dichlorophenoxy)-ethylamine (2,4-D ethylamine)

2,4-Dichlorophenoxy-acetaldehyde

2,4-D (herbicidal)

Scheme 2

That the amine oxidation is dependent on the presence of an amine oxidase has been demonstrated by isolating the enzyme from pea seedlings and effecting the conversion *in vitro*. Furthermore, the herbicidal effects produced by treating plants with 2,4-D ethylamine do not appear if the plants are pretreated with the amine oxidase inhibitor 2-hydroxyethylhydrazine.[27]

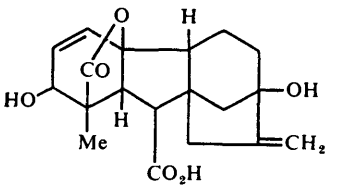
With some phenoxy-acid herbicides, selectivity can arise not from differential lethal synthesis but because they are more readily degraded to inactive products in one species than in another. An example of this is mecoprop, 2-(2-methyl-4-chlorophenoxy)propionic acid, first prepared in the reviewer's laboratory in 1953.[28] The racemic form of this substance was shown to be as active as 2-methyl-4-chlorophenoxyacetic acid (MCPA) in four different tests for plant growth-regulating activity. Lush[29] and Leafe[30] demonstrated that mecoprop also controls two weeds, chickweed (*Stellaria media*) and cleavers (*Gallium aparine*), which are not well controlled by MCPA. Evidence obtained from radioactive tracer studies indicates that the resistance of cleavers to MCPA is due to a detoxification within the tissues whereby both carbon atoms of the side-chain are lost.[31] The steric effect of the α-methyl group in mecoprop, however, protects against this degradation, enabling the compound to accumulate in the tissues and produce its

[25] R. L. Wain, *Agriculture*, 1957, **63**, 575.
[26] 'Weed Control Handbook', ed. J. Fryer and R. Makepeace, Blackwell Publications, Oxford, 1970.
[27] R. J. Nash, T. A. Smith, and R. L. Wain, *Ann. Appl. Biol.*, 1968, **61**, 481.
[28] C. H. Fawcett, D. J. Osborne, R. L. Wain, and R. D. Walker, *Ann. Appl. Biol.*, 1953, **40**, 232.
[29] G. B. Lush, Proceedings of the IIIrd British Weed Control Conference, Blackpool, 1956, British Weed Control Council, London, p. 625.
[30] E. L. Leafe, ref. 29, p. 633.
[31] E. L. Leafe, *Nature*, 1962, **193**, 485.

physiological effects. MCPA is also subject to other types of chemical modification and indeed most herbicides are metabolized at varying rates in plants. Decarboxylation, hydrolysis, dealkylation, hydroxylation of ring, conjugation, and ring cleavage are all known to occur. When breakdown occurs to a greater extent in one plant species than another, we have a basis for herbicidal selectivity.

3 The Gibberellins

The second group of endogenous plant growth hormones are the *gibberellins*. This area of research began with the discovery by Kurasawa in 1926 that the cell-free sterile filtrate of a fungus *Gibberella fujikuroi*, which causes pale spindly growth of rice, produced marked growth stimulation when applied to seedlings of rice and grasses.[32] Gibberellins occur in all higher plants and some 52 of them have now been characterized; they differ only slightly in structure from the well known gibberellic acid GA_3 (11) which is produced commercially from fungal cultures.



(11) Gibberellic acid GA_3

Like auxins, gibberellins promote stem extension and fruit growth. They can also stimulate flowering in some plants and overcome dormancy of certain seeds. Gibberellins promote fruit setting and the growth of seedless grapes and improve skin quality and delay the ripening of citrus fruits. GA_3 is also used in the brewing industry to stimulate the production in barley of the enzyme α-amylase, which plays a key role in the breakdown of starch during malting.

Recent research has led to the discovery of a number of synthetic compounds which appear to act by inhibiting gibberellin biosynthesis. A plant so treated must therefore depend mainly on its auxin for extension growth and so it will be retarded. Not all growth retardants work in this way but by their use it is possible to obtain dwarfed plants with shortened internodes. Such plants are sturdy and healthy with deep green leaves and in some cases they have been found to have greater pest and disease resistance.

Most of the many growth retardants synthesized in our laboratory are ammonium, phosphonium, or sulphonium salts;[33] 4-chlorobenzyltri-n-butyl-

[32] E. Kurosawa, *J. Nat. Hist. Soc. Formosa*, 1926, **16**, 213.
[33] B. E. A. Knight, H. F. Taylor, and R. L. Wain, *Ann. Appl. Biol.*, 1969, **63**, 211.

ammonium bromide, for example, is very effective for dwarfing important legume crops such as French bean (*Phaseolus vulgaris*) and soya bean (*Glycine max*), as well as certain ornamentals, when sprayed on to the young plants at 500—1500 p.p.m. (Plate 3). The dwarfed plants which result from these treatments take up less space so that more plants per acre can be grown. Whether or not this leads to higher yields per acre is now under investigation. Some of our other compounds, *e.g.* *N*-methyl-*N*-chloromethylpyrollidinium bromide, are highly effective in dwarfing wheat and oats,[34] as is the commercial product chlormequat (2-chloroethyltrimethylammonium chloride).

Dwarf wheat varieties can also be obtained by breeding; this has been achieved at the Plant Breeding Institute at Cambridge, and Borlaug's now famous dwarf high-yielding wheat varieties in which the Japanese Norin 10 dwarfing gene was incorporated into Mexican wheats are playing an important part in the 'Green Revolution'. Recent work in the reviewer's laboratory has indicated that the dwarfing effect which operates in such genetic dwarfs may arise from the synthesis of hormone-inhibitory substances within the plant. The compounds responsible, which would appear to be 'natural' growth retardants, are now being intensively studied.

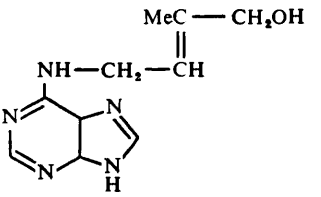
A well known commercial synthetic growth retardant is *NN*-dimethylamino-succinamic acid, which, among other uses, has been shown to reduce extension growth and to promote flowering in apple trees.

4 Cytokinins

As already stated, auxins and gibberellins are hormones which promote cell enlargement. The other fundamental process which determines growth is cell division by which new cells are produced. The hormones which can influence this process in plants are known as the cytokinins. The first compound possessing cytokinin activity to be discovered was 6-furfuryladenine (kinetin), isolated in 1955 from autoclaved herring sperm DNA.[35,36] However, it was not until 1964 that the first naturally occurring cytokinin, zeatin, was reported. This was found by Letham[37] in developing maize kernels and like kinetin it is a 6-substituted adenine derivative [6-(4-hydroxy-3-methylbut-2-enyl)aminopurine (12)].[38] Zeatin and its riboside occur widely in plants.

Cytokinins can delay senescence. A detached radish leaf, for example, spot-treated with a cytokinin, remains green in the treated area when the rest of the leaf becomes yellow.[39] Furthermore, it has been shown that a ^{14}C-labelled amino-acid applied to the non-treated area of the leaf migrates to the site of cytokinin

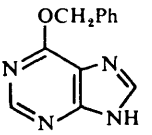
[34] V. K. Chamberlain, K. Chamberlain, and R. L. Wain, *Ann. Appl. Biol.*, 1976, **82**, 589.
[35] C. O. Miller, F. Skoog, M. H. von Saltza, and F. M. Strong, *J. Amer. Chem. Soc.*, 1955, **77**, 1392.
[36] C. O. Miller, F. Skoog, F. S. Okumura, M. H. von Saltza, and F. M. Strong, *J. Amer. Chem. Soc.*, 1955, **77**, 2662.
[37] D. S. Letham, *Life Sci.*, 1963, **2**, 569.
[38] D. S. Letham, J. S. Shannon, and I. R. McDonald, *Proc. Chem. Soc.*, 1964, 231.
[39] A. E. Richmond and A. Lang, *Science*, 1957, **125**, 650.



(12) Zeatin

treatment;[40] this indicates that the chemical is promoting normal metabolism at the expense of the rest of the senescing leaf. Cytokinins therefore have potential for promoting the life of fresh vegetables such as lettuces and cabbages. They can also be used for promoting the life of cut flowers and overcoming the dormancy of certain seeds. Cytokinins appear to be synthesized in the roots of plants.

Many 6-substituted adenine derivatives have been tested for cytokinin activity.[41] A useful test is to use explants of tobacco pith tissue on sterile agar medium. The control agar is provided with all the necessary nutrients, mineral elements, and growth substances except cytokinins; since cell division cannot take place no growth occurs. When the agar contains cytokinin, however, the cells do divide and then enlarge, thereby leading to the growth of undifferentiated callus tissue. In recent research carried out in the reviewer's laboratory, cytokinin activity has been found in a range of 6-substituted oxypurines. When examined in the tobacco pith test some of them [*e.g.* 6-benzyloxypurine (13)] not only promote callus growth but this then undergoes morphological differentiation leading to the production of intact tobacco plants[42] (Plate 4).



(13) 6-Benzyloxypurine

As we have seen, natural cytokinins operate with auxins and gibberellins in the hormonal complex which controls growth and development. In an attempt to determine whether, in the growth of the wheat coleoptile, these operate together or in sequence, a study was made of the changes in the protein (and enzyme) pattern at different stages of growth.[43] Protein fractions were taken from coleoptiles at all growth stages and antiserum was raised from the combined fractions in

[40] K. Mothes, 'Régulateurs Naturels de la Croissance Végétale', Paris, Edition du C.N.R.S., 1964, p. 131.
[41] K. Rothwell and S. T. C. Wright, *Proc. Roy. Soc.*, 1967, **B167**, 202.
[42] E. J. Wilcox and R. L. Wain, *Ann. Appl. Biol.*, 1976, **84**, 403.
[43] S. T. C. Wright, *Symp. Soc. Exp. Biol.*, 1963, **17**, 18.

the rabbit. Using a technique involving the diffusion, in an agar gel, of protein extracts of coleoptiles of different ages and their antibodies, it was shown that qualitative changes in protein occurred during growth and cellular differentiation of the wheat coleoptile. It was logical therefore to expect the responses of the coleoptiles to gibberellin, kinetin, and IAA to vary at different stages of growth. This was found to be the case; there were clear indications that these substances exert their growth effects by acting in a well defined sequence.[44] The first early phase of development was one of cell enlargement (approximately 0—30 h after sowing) influenced mainly by GA; the second (approximately 30—60 h after sowing) in which many of the cells were undergoing cell division was influenced mainly by kinetin. The third phase was a final period of cell enlargement (approximately 60—120 h after sowing) which was promoted mainly by IAA. It has since been postulated that these three classes of growth regulator may operate in a similar sequential manner in the growth of fruit.[45] Furthermore, it has been shown that tobacco pith tissue grown in a nutrient medium under sterile conditions also appears to have a sequential requirement for GA, kinetin, and IAA.[46]

5 Ethylene

At this stage, mention should be made of another compound which can exert profound physiological effects on plants. This is the simple unsaturated, gaseous hydrocarbon *ethylene*. It is evolved by certain plants and especially by ripening fruits, and it can promote abscision of both fruit and leaves. Much work has been carried out on the mode of action of ethylene, the specific effects of which must be related to its unsaturated character and its small molecular size, since higher olefins are much less active and the corresponding paraffin, ethane, is inactive.

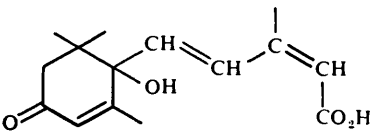
A compound which breaks down to yield ethylene following application to plant tissues has interesting commercial uses. This compound is ethephon, 2-chloroethylphosphonic acid. One striking effect which it produces is to stimulate greatly the flow of rubber latex when applied to rubber trees on a band of smoothed bark below the tapping cut. It is also used for accelerating maturity and early ripening of tomatoes and citrus fruits and for inducing uniform flowering in pineapples.

6 Hormone Inhibitors

The three main types of hormone so far discussed are concerned with the promotion of growth. It has long been suspected, however, that inhibitory compounds might operate in growth control processes as, for example, when, owing to unfavourable environmental conditions, growth ceases altogether. In 1965 a naturally occurring hormone inhibitor was isolated from cotton fruits and identified as 3-methyl-5-(1-hydroxy-4-oxo-2,6,6-trimethylcyclohex-2-en-1-yl)-

[44] S. T. C. Wright, *Nature*, 1961, **190**, 699.
[45] J. Van Overbeek, *Proc. Campbell Soup Co. Pl. Sci. Symp.*, 1962, 38.
[46] J. P. Nitsch, 'Biochemistry and Physiology of Plant Growth Substances', ed. F. Wightman and G. Setterfield, Runge Press, Ottawa, 1968, p. 563.

cis,trans-penta-2,4-dienoic acid (14).[47] Shortly afterwards the same compound was found in sycamore leaves and in lupin pods. The synthesis of this inhibitor, which has been given the name *abscisic acid*, was achieved in Cornforth's laboratory.[48]



(14) Abscisic acid

Abscisic acid has since been found in a wide range of plant species, and physiological studies have revealed that it can inhibit the activity of auxins, gibberellins, and cytokinins. Not only this, but we have also shown that abscisic acid operates in defending plants against the effects of physiological stress.[49,50] For example, when water is withheld from a tomato plant the wilting plant responds by producing up to fifty times the normal level of abscisic acid in its leaves (Plate 5). The effect of this is two-fold. Firstly, the resulting inhibition of growth hormone activity stops the plant from growing and energy is thereby conserved; secondly, a closure of the leaf stomata is induced[51] and water loss by transpiration is cut down. By two mechanisms therefore the build up of endogenous abscisic acid provides the plant with a better chance of survival during the drought period.

In our laboratory we have compared the capacity of a Mexican drought-resistant maize variety ('Latente') with two others which are not drought resistant.[52] It was found that when subjected to standard water stress conditions 'Latente' produced much more abscisic acid than the other two varieties, again indicating the important role played by this inhibitor in plants subjected to drought. A similar rapid increase in abscisic acid levels has also been shown to occur when plant roots are waterlogged (Figure, opposite p. 275).[53]

Abscisic acid therefore provides a defence mechanism against physiological stress. In addition to the free acid, abscisyl β-D-glucopyranoside has been isolated from plant tissues.[54]

A feature of the abscisic acid molecule is its resemblance to Vitamin A, a substance which is produced in the animal liver from certain carotenoid pigments supplied in the food. Although carotenoids are present with chlorophyll in all

[47] K. Okhuma, F. T. Addicott, O. E. Smith, and W. E. Thiessen, *Tetrahedron Letters*, 1965, **29**, 2529.
[48] J. W. Cornforth, B. V. Millborrow, and G. Ryback, *Nature*, 1965, **206**, 715.
[49] S. T. C. Wright, *Planta*, 1969, **86**, 10.
[50] S. T. C. Wright and R. W. P. Hiron, *Nature*, 1969, **224**, 719.
[51] R. J. Jones and T. A. Mansfield, *J. Exp. Bot.*, 1970, **21**, 714.
[52] A. Larqué-Saavedra and R. L. Wain, *Nature*, 1974, **251**, 716.
[53] R. W. P. Hiron and S. T. C. Wright, *J. Exp. Bot.*, 1973, **24**, 769.
[54] K. Koshimizu, M. Iniu, M. Fukui, and T. Mitsui, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 789.

green leaves, and therefore occur abundantly throughout the plant kingdom, their physiological function within the plant has never been properly elucidated.

However, the similarity between Vitamin A and abscisic acid led to speculations in our laboratory[55] on whether carotenoids might serve as precursors of abscisic acid within the plant. Since plants in the dark grow taller than those in the light, it follows that light inhibits growth. Light therefore might be a factor which could promote the conversion of carotenoid into the inhibitor abscisic acid. This reasoning agreed with one of our earlier findings[56] that a growth inhibitor is produced in dwarf pea plants when they are exposed to white light.

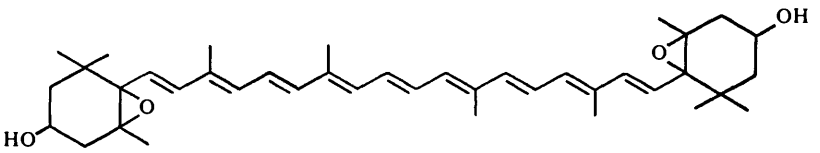
To test the above hypothesis, equal volumes of an acetone solution of nettle leaf carotenoids were applied uniformly to two filter papers and the solvent was evaporated. One of these papers was kept in darkness and the other was exposed to the light for 1 hour; they were then placed in Petri dishes, moistened with water, and each was sown with cress seeds. The dishes were then placed in an incubator. After 60 hours in the dark it was found that all the seeds on the paper which had been kept in the dark had germinated whereas no germination had occurred on the paper which had been exposed to the light (Plate 6). Thus, simple exposure of the mixed carotenoids to light had led to the formation of a potent seed-germination inhibitor.[55] However, further investigation showed that the inhibitor was a neutral substance; it was therefore not abscisic acid.

Examination of a range of pure carotenoids by the above procedure revealed that the precursors of the inhibitor were certain xanthophylls of which the most important is the epoxide violaxanthin (15).[57] Large quantities of this pigment were extracted from orange peel. When photo-oxidized three main products (16)—(18) were identified. Of these, one showed growth inhibitory activity fully comparable with that of abscisic acid.[58] It was a mixture of the 2-*cis*-4-*trans*- and 2-*trans*-4-*trans*-isomers of 5-(1,2-epoxy-4-hydroxy-2,6,6-trimethyl-1-cyclohexyl)-3-methyl-pentadienal. These geometric isomers were separated and most of the inhibitory activity was found to reside in the 2-*cis*-4-*trans*-isomer. In view of its formation by the oxidation of certain xanthophyll pigments this new inhibitor has been given the name xanthoxin.[59] That the inhibitor is formed within the intact plant has also been established; pea seedlings, for example, exposed to short intervals of light contain some seven times more xanthoxin than similar plants held in the dark.[60]

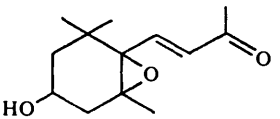
Thus, in xanthoxin we have another naturally occurring inhibitor which operates in the chemical control of plant growth. Its formation from xanthophyll epoxides offers for the first time an explanation of why plants grow taller in the dark. Not only this, but the bending of plants towards the light may depend at

[55] H. F. Taylor and T. A. Smith, *Nature*, 1967, **215**, 1513.
[56] G. M. Simpson and R. L. Wain, *J. Exp. Bot.*, 1961, **12**, 207.
[57] H. F. Taylor and R. S. Burden, *Proc. Roy. Soc.*, 1972, **B180**, 317.
[58] H. F. Taylor and R. S. Burden, *Phytochemistry*, 1970, **9**, 2217.
[59] H. F. Taylor and R. S. Burden, *Nature*, 1970, **227**, 302.
[60] R. S. Burden, R. D. Firn, R. W. P. Hiron, H. F. Taylor, and S. T. C. Wright, *Nature*, 1971, **234**, 95.

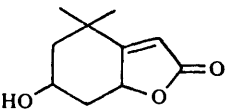
least in part upon the production of the inhibitor on that side of the plant which is exposed to the light.



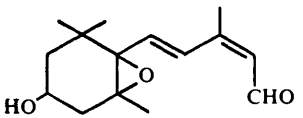
(15) Violaxanthin



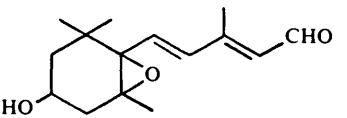
(16) Inactive



(17) Inactive



2-*cis*-4-*trans*-Xanthoxin



2-*trans*-4-*trans*-Xanthoxin

(18)

7 Promotion of Root Growth

So much then for the hormones and hormone inhibitors which, in the light of present knowledge, control the growth of plants. What about that vital part of the plant which is not normally seen—the roots growing in the soil? Research on hormones in relation to root growth has so far not been extensive and, indeed, it is only recently that indole-3-acetic acid has been unequivocally shown to operate in roots. In some of our recent experiments we have demonstrated that root growth is restricted on exposure to light, that the light receptor area is the root cap, and that with some species only a short flash of light is enough to produce an inhibitory effect.[61–63]

An interesting development in this area of research came unexpectedly when we were examining the biological activity of 3,5-di-iodo-4-hydroxybenzoic acid

[61] H. Wilkins, R. S. Burden, and R. L. Wain, *Ann. Appl. Biol.*, 1974, **78**, 337.
[62] H. Wilkins and R. L. Wain, *Planta*, 1975, **123**, 217.
[63] H. Wilkins, A. Larqué-Saavedra, and R. L. Wain, 'Plant Growth Substances', Hirokawa Publ. Co., Tokyo, 1973, p. 1231.

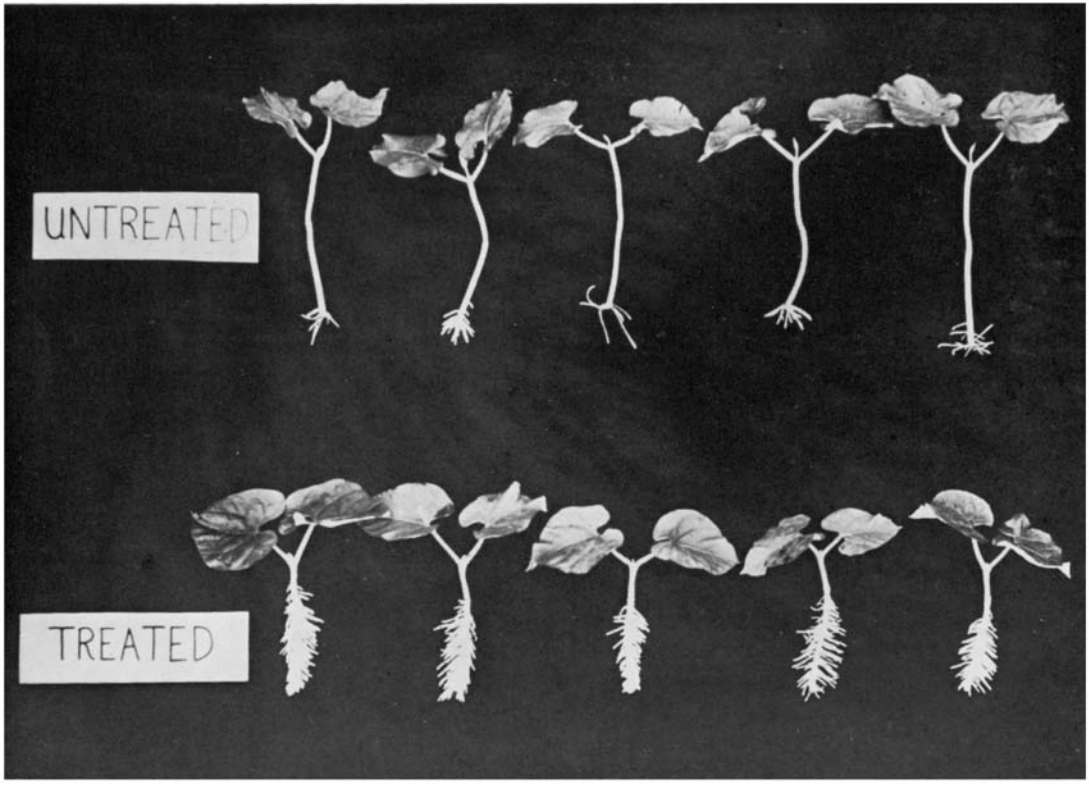


Plate 1 *Effect of indole-3-acetic acid in promoting rooting. Top row: Bean cuttings rooting in water through action of auxin moving down stem from the growing tip. Bottom row: Similar cuttings placed in solution containing* 50 *p.p.m. indole-3-acetic acid*

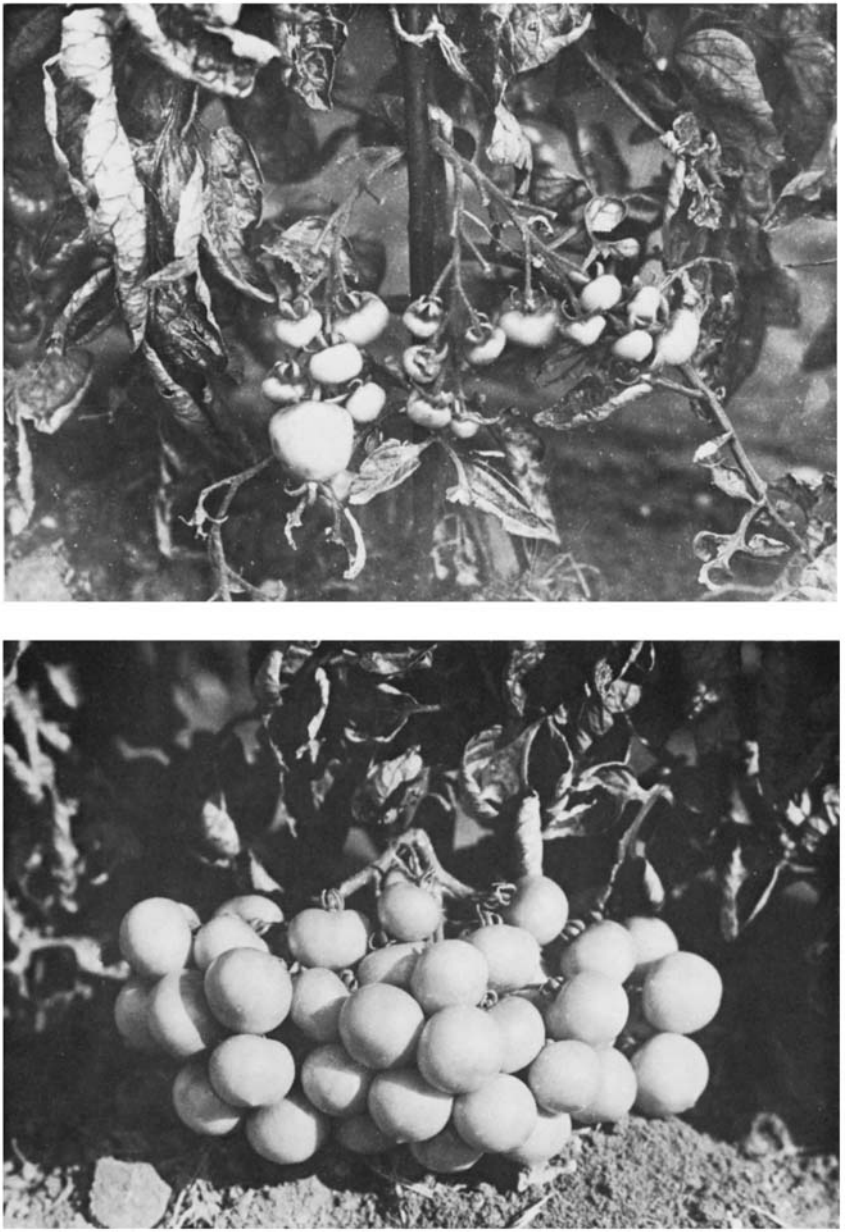


Plate 2 *Setting tomatoes. Top*: *Untreated bottom truss of outdoor tomato plant. Bottom*: *Similar plant of which the bottom flower truss had been sprayed with* α-(2-*naphthoxy*)-*propionic acid at* 100 *p.p.m. in water.*

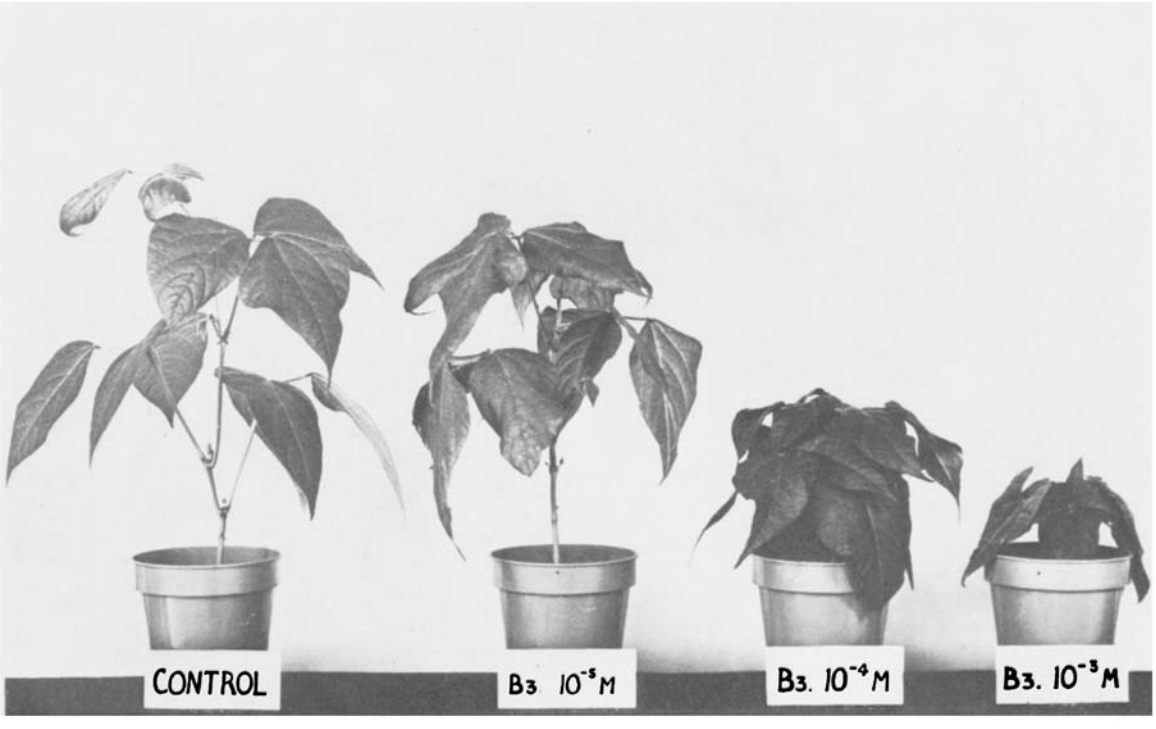


Plate 3 *Effect of spraying the Wye retardant* 3-*chlorobenzyltributylammonium bromide at* 10^{-5}, 10^{-4}, *and* 10^{-3} mol l^{-1} *on to French bean plants*

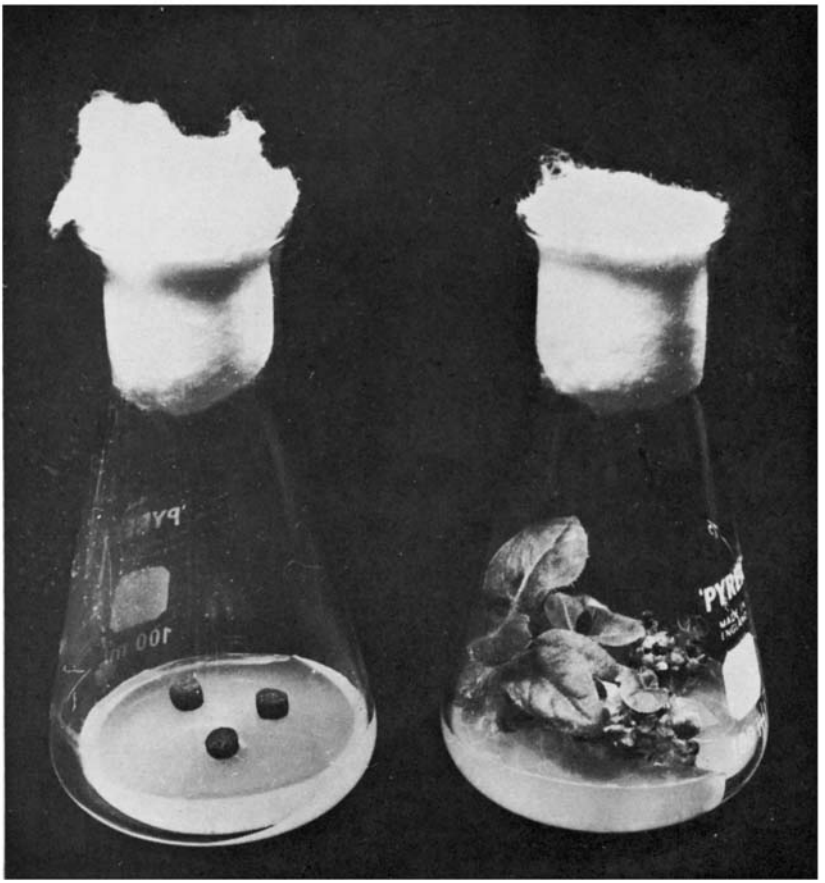


Plate 4 *Cytokinin activity of* 6-*benzyloxypurine. Tobacco pith segments on left fail to grow on nutrient medium containing no cytokinin. In flask on right containing the oxypurine, growth has occurred and morphological differentiation has also taken place with the production of young plants*

Plate 5 *Tomato plant on left supplied with adequate water has only one unit of abscisic acid in its leaves. In response to water stress the leaves of the wilting plant on right have built up 52 times this amount of the inhibitor*

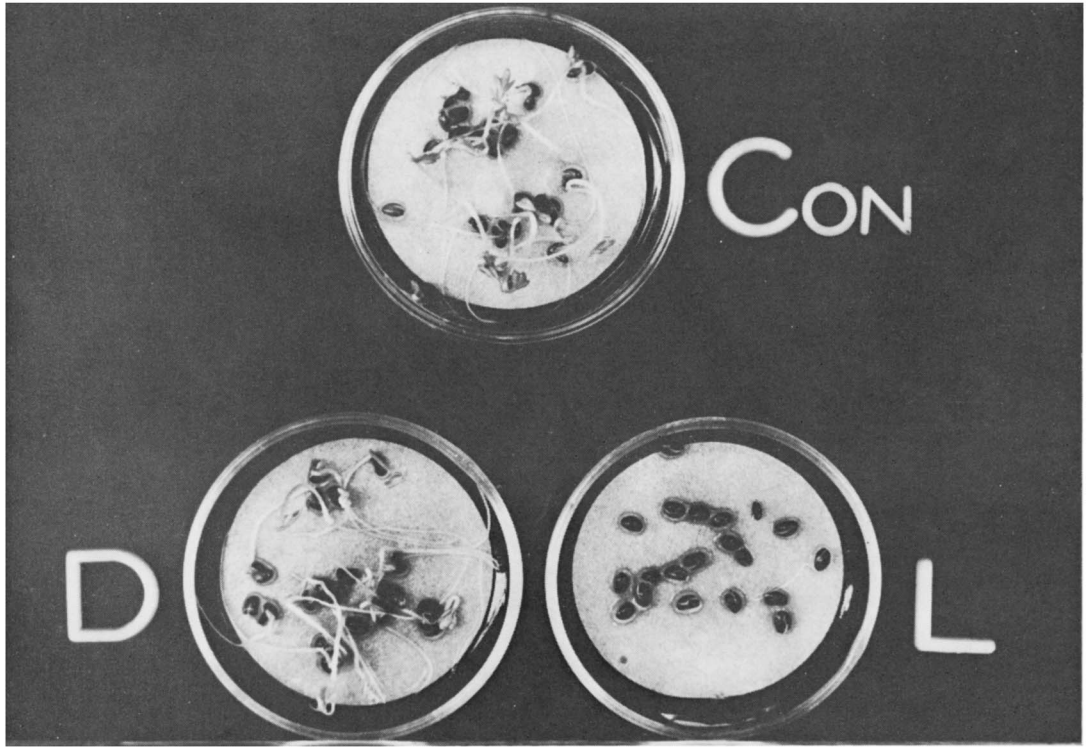


Plate 6 *Production of a seed germination inhibitor on exposure of carotenoid pigments to light. Carotenoid-treated paper in dish on left which had been held in the dark permits* 100% *germination of cress seeds whereas a similar paper which had previously been exposed to light allows no germination. Top dish shows germination on untreated paper*

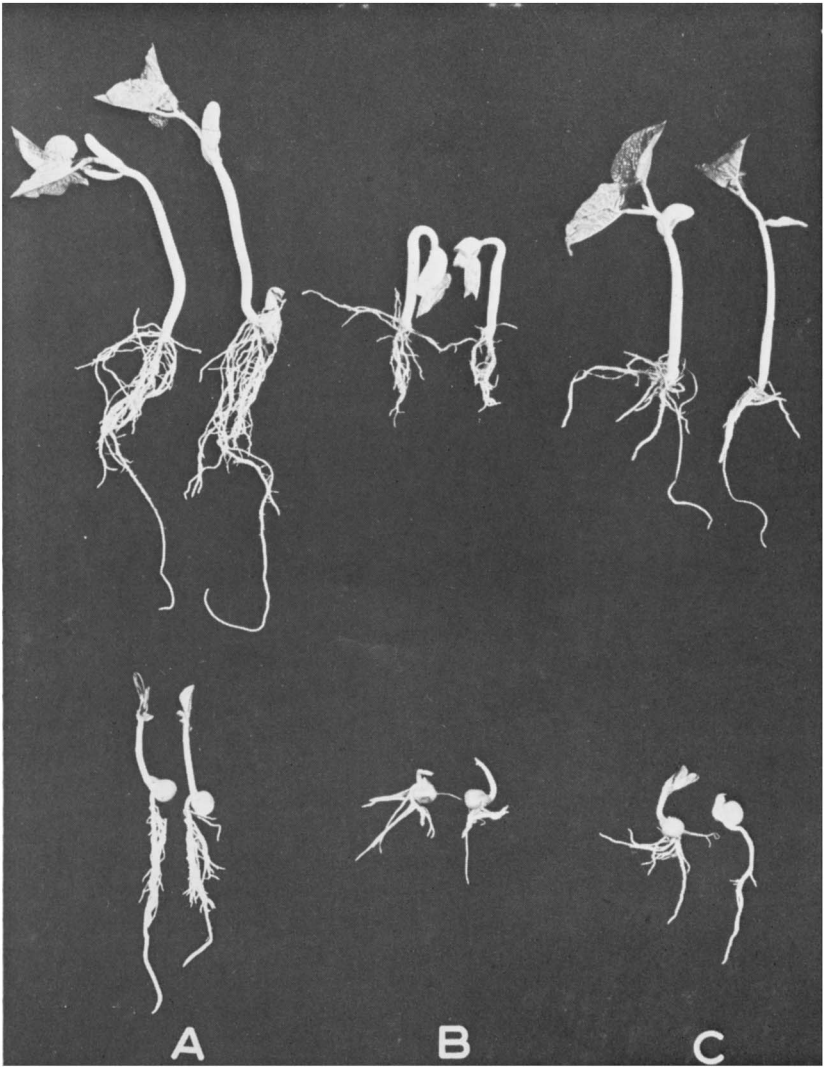


Plate 7 *Effect of DIHB in promoting root growth of bean seedlings (top row) and pea seedlings (bottom row). Plants on right show growth in loose soil: those in middle, plants in compacted soil; those on left, plants in compacted soil treated with* 10^{-5} mol l^{-1} *DIHB*

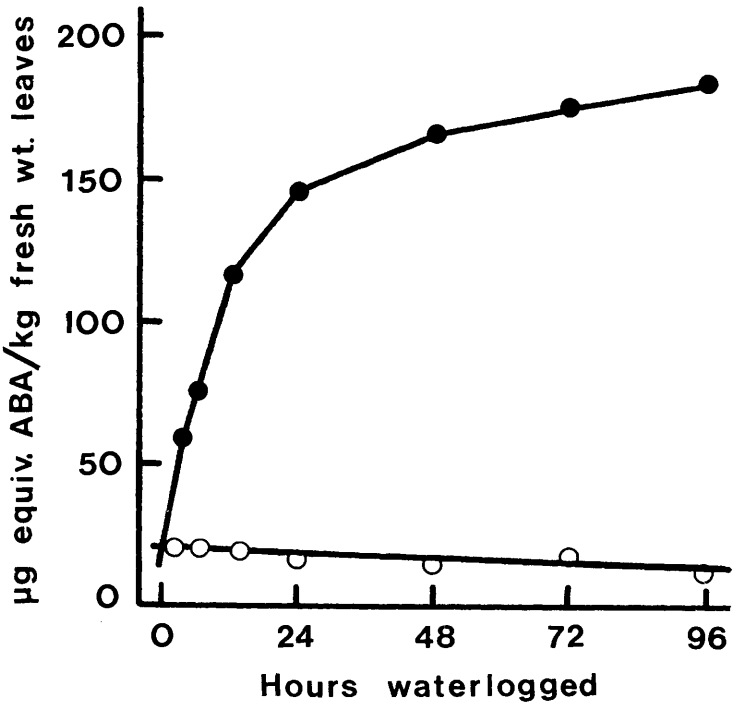
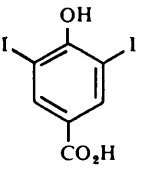
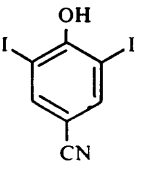


Figure *Levels of abscisic acid (ABA) in dwarf bean seedlings:* ● = *soil waterlogged*; ○ = *soil not waterlogged*

(DIHB) (19), a compound closely related to ioxynil (20), a selective herbicide discovered in the reviewer's laboratory.[64] When cress or rice seedlings were grown in a normal culture solution, roots exposed to light were found to grow to only



(19) DIHB



(20) Ioxynil

one-third the length of those growing in the dark. However, this inhibitory effect was completely removed when DIHB was present in the solution at 10^{-5} mol l^{-1}. This effect can be spectacular; with seedlings of cress (*Lepidium sativum*), for example, roots standing in a 10^{-5} mol l^{-1} solution of DIHB grow three times longer than those not receiving the treatment.[65] Thus, DIHB removes a constraint on root growth which is imposed by exposure to light.[66]

Unfortunately, roots in the soil are in the dark so the above beneficial response from DIHB treatment cannot be exploited. However, other constraints on the growth of roots can operate in the field, such as mechanical impedence. When this occurs, DIHB treatment has been shown to have a beneficial effect on root growth.[67] This is illustrated for compacted soils in Plate 7. The agricultural implications of these findings and studies on the mode of action of DIHB are now being intensively examined.

In this Review I have endeavoured to show that research on hormones and inhibitors is not only providing a better understanding of plant growth but that it is leading to agricultural developments and increased food production. The biological properties of these unique organic molecules would surely have interested John Jeyes had he been with us here today. At any rate, I would like to think so.

[64] R. L. Wain, *Nature*, 1963, **200**, 28.
[65] R. L. Wain, H. F. Taylor, P. Intarakosit, and T. G. D. Shannon, *Nature*, 1968, **217**, 870.
[66] R. L. Wain, P. Intarakosit, and H. F. Taylor, *Mededel. Rijk. Land. Gent*, 1968, **33**, 1341.
[67] S. M. Wilkins, H. Wilkins, and R. L. Wain, *Nature*, 1976, **259**, 329.