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Progress in our Understanding of Plant Growth

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Abstract

EFFORTS to improve plant production are increasingly dependent on a better understanding of plant metabolism and growth. A summary is given of the present state of our knowledge in some branches of plant physiology. Recent work in mineral nutrition suggests that more than the usually accepted 13 elements may be essential for growth. There is some evidence that particular plants may require elements not generally essential. The processes of transport of nutrients in the plant are proving more complex than hitherto believed. Thus in the xylem stream there is movement of some organic compounds as well as of inorganic ions. The movement of calcium in the phloem appears to be greatly restricted, leading to localised deficiencies of this element in some developing organs. Four distinct types of plant hormones have now been recognised in many plants. These compounds have characteristic biological activity and are responsible for the stimulation or inhibition of many aspects of plant growth. Plants are now known to possess methods for regulating their metabolism. Thus at least two enzymes—nitrate reductase and urease—are known to be induced enzymes, that is, are produced only when the appropriate substrate is present.

INTRODUCTION

GREEN plants provide the basis for all life on this planet. This fact alone would appear to provide sufficient justification for talking about the growth of green plants. The immediate dependency of the economy of this country on the satisfactory growth of plants would seem to give additional relevancy to the subject here. Yet, in the scientific atmosphere of today, I feel some justification is necessary. A recent article in *Nature* (220, 521: 1968) entitled "Is Botany Dead?" summarised various doubts held by some people about the current scientific status of the study of plants.

In the past in the development of biology, research with higher plants has made notable contributions to fundamental knowledge. When Mendel did his original genetic work it was perfectly natural for him to work with a higher plant, the pea plant. Later, in this century, the first enzyme to be obtained in pure crystalline form from any living organism was obtainable from the seeds of a bean. Again the first virus to be isolated from any type of organism was obtained from tobacco plants. In more recent years much of the work in the field of biochemistry has been with micro-organisms or animal tissues. In particular, most of the more

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spectacular developments of molecular biology since 1950 have resulted from work with bacteria. A consequence of this has been a continuing decrease in incentive for work on the physiology and biochemistry of higher plants despite their importance to man.

However, although the total research effort on plants has been relatively small, there have been recent advances in our understanding of plant growth and nutrition. In this talk I would like to attempt to summarise the state of our knowledge of certain parts of this field, to say something of our increasing understanding of plant growth, of our attempts to explain the various phenomena of growth and metabolism in physiological and biochemical terms. What I have to say will be in the nature of progress reports on a number of topics, in which we have been interested, rather than a systematic account of completed research.

It should be appreciated that these organisms we speak of as green plants are a highly varied group of organisms. There are about 130,000 species of higher green plants. Bailey's " Manual of Cultivated Plants " lists about 3,600 species as cultivated to some degree by man, but of these only about 150 are extensively cultivated. Plant species vary widely in size, shape, and habit. Some are herbs, some shrubs, some trees; some are adapted to life in the tropics, some to the arctic; some to water, some to the desert. Some species are cultivated for their leaves, some for their seeds, some for their fruits, and some for their fibres or wood. Let us look for a moment at two types of green plant, extremes as far as general morphology and organisation are concerned:

1. *Large trees*: There are many species of large tree. The largest are the California *Sequoias*. These have two biological distinctions. They are probably the largest living things (known to stand over 300ft high with a 35ft diameter) and the oldest living things (over 3,000 years old).

2. *Duckweeds*: These are the simplest of the higher plant species—the family Lemnaceae with genera *Lemna, Spirodela,* and *Wolffia*. These are simple, small, water plants consisting of a single small green leaf (about 2–3mm long) which may or may not bear very simple roots.

These two disparate sorts of plant present different opportunities to the experimental physiologist. For example the size of trees allows one to extract sap from individual branches in quantities which can be analysed, and in this way to study the nutrients being transported around the plant. In contrast the duckweeds because of their size can be grown in sterile culture in the laboratory—much as a microbiologist grows a bacterial or fungal culture. This ability allows the physiologist to study their nutrition in detail not possible with other types of plant—for example the ease with which culture solutions can be changed allows study of the ability of plants to adapt to changing conditions.

In this talk I do not want to deal with the individual characteristics of any one type of plant but to deal with a number of aspects of plant growth that may be relevant to plants in general. I will first of all say something about the nutrition of plants, something of our efforts to define those materials that the plant must acquire from its environment. Then I would like to discuss the movement of these materials within the plant body and the inter-relationships of the different plant organs. Finally I would like to talk about the ways in which regulation of the multiple activities that occur in the plant body is brought about. I will say something about hormonal control of plant growth and something about the way in which the various metabolic reactions appear to be regulated.

MINERAL NUTRITION OF PLANTS

The outstanding characteristic of green plants is unquestionably their synthetic capacity. From a number of relatively simple materials acquired from the environment plants build up all that complex of chemical compounds that goes to make the substance of the plant body. In this regard plants are of course much more versatile than the animal body, which is completely dependent on many materials already synthesised by plants. It has been the first task of those concerned with plant nutrition to attempt to define precisely what materials of the environment are essential for plant growth.

First in the list of essentials come water and carbon dioxide. These requirements have been long recognised. Over 200 years ago Priestley knew that green plants, in the presence of light, could chemically fix the carbon dioxide of the air. It gradually came to be realised that all the carbon of the plant body comes from this source, and, of course, because animals are nutritionally dependent on plants, all the carbon of the animal body must also come from this one process.

Plants also require certain mineral elements, which they normally obtain from the soil. Plant tissue is known to contain most of the chemical elements found in the soil, but early workers in plant nutrition soon appreciated that not all of these elements were essential for plant growth. Some elements appeared to be there because they occurred in the soil and were taken up by the plant along with those elements essential for growth. Definition of which elements were in fact essential became the first major question to which workers in the field of plant mineral nutrition addressed themselves.

About 100 years ago there was general belief that only seven elements were necessary for normal plant growth. These were nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and iron. The experiments from which this belief arose consisted simply of growing plants in solution or sand culture completely away from soil. The composition of the culture solutions supplied to the plants was thought to be known completely. From such experiments it appeared that plants supplied with only the above seven elements made as good growth as plants growing under the most favourable conditions in soil.

Not until about 1900 was it realised that other elements were also necessary for plant growth. This occurred only when better methods became available for purifying the salts and water used in such experiments. Using such specially purified salts various workers then showed that manganese and zinc were also necessary for plant growth. It appeared that earlier workers had unknowingly included these elements in their nutrient solutions either as impurities in the salts or water used. Four other elements were then shown to be necessary for growth of plants. In 1923 boron was added to the list. In 1931 copper, in 1939 molybdenum, and as late as 1954 chlorine were shown to be essential. At this stage then 13 elements appeared to be essential (Table I) and this is the situation as still described in most text books.

TABLE I.—Average Content of Essential Elements in Dried Plant Material.

	percent	p.p.m.
Nitrogen	2	20,000
Potassium	1.5	15,000
Calcium	1.5	15,000
Magnesium	0.3	3,000
Phosphorus	0.25	2,500
Sulphur	0.2	2,000
Chlorine		250
Iron		100
Boron		40
Manganese		40
Zinc		40
Copper		25
Molybdenum		1 or less

Is this list of 13 elements complete, or is it possible that other elements may be essential for growth? In one respect this question is technically difficult to answer. The molybdenum requirement for plant growth is very small, and with this element we are getting near the practicable limits of detection and purification. Other elements present in similar or lesser amounts may well also be essential. For example there is some recent evidence that the element cobalt may be necessary for plant growth. This element has long been known to be necessary in animal nutrition but has been considered to be of no significance for plants. The new evidence now suggests that it is required by plants at a very low level. There is also evidence that the element vanadium may be required by plants.

We are also being forced to the conclusion that there are some elements that may be required by only some plants, in addition to those already considered to be universally required. It has been shown for example that the element sodium is required in relatively large amounts for the growth of some species of the genus *Atriplex*. It has also been shown that the element silicon is essential for rice plants and may well be required by other grass species. This concept is new in plant nutrition, and it will be most interesting to see how far we have to extend our ideas in the coming years.

We are also being forced to modify our ideas of essentiality and non-essentiality. For example some apparently non-essential elements may be always present in plants and may function in some quite precise way in plant metabolism. Thus the elements selenium, iodine, or fluorine occur regularly in some plants and are regularly metabolised forming quite specific organic compounds. Then again elements closely related to some of the essential elements may be in some degree capable of replacing them. The best known example is probably the ability of strontium to replace calcium to some extent. The element strontium to the best of our knowledge is not required for the growth of plants, but if strontium is supplied then apparently less calcium is required. It would seem that to some extent strontium can perform some of the tissue functions of calcium.

The work of defining the elements required by plants has occupied much of the attention of plant nutritionists up to the present time. However, other problems in plant mineral nutrition are now becoming apparent. We have little understanding of many of these and there is yet little that can be said except to attempt to define some of them:

1. There is growing interest in differences shown by plants with regard to their mineral nutrition. It is often claimed that legumes contain more than twice as much calcium as grasses. Even when clovers are growing closely associated with grasses, the clover foliage will contain perhaps 2 percent calcium while the grass foliage contains less than 1 percent. The reasons for these differences are not clear: whether they reside in the ability of the various species to take up nutrients from the same environment, or whether the tissues of some species require higher levels than other species. It is also known that there are wide differences between plants in their ability to grow in a phosphate-deficient soil. In such a soil some species of plants will grow well while other species will scarcely remain alive. The latter will grow adequately if phosphorus is supplied. Again there is little knowledge of the physiological basis of these differences between species.

2. An extreme case of species differences occurs with the so-called indicator or accumulator species. These are species of plant which normally contain particular elements at unusually high levels. The elements may be essential nutrient elements such as molybdenum or zinc, or elements not considered necessary for plant growth such as nickel or even gold and uranium. There is some practical interest today in biogeochemical prospecting, whereby analyses of plants growing in certain areas are used in seeking the location of ores rich in particular elements. But the plant physiologist has yet little understanding of this remarkable phenomenon.

3. Man frequently wants to grow plants in unfavourable mineral environments. For example the soil may contain too much salt. There are plants (so-called halophytes) which normally grow in saline environments, but among the ordinary

run of crop plants there are marked differences in ability to grow in a partially saline environment. This phenomenon is recognised by those concerned with reclamation of marine areas, where it is well known that only certain species may be grown in the earlier stages of reclamation. Again, the soil may be unfavourable by being too acid or too alkaline. Some plants favour acid growing conditions, others more alkaline conditions. Plant ecologists have long recognised some species of plants as calciphiles, or lime-loving species, while other species are calciphobes, or lime-hating species. Different species of the one genus may differ in this way, and differing ecotypes of the one species have also been found. A third type of unfavourable mineral environment occurs where there is something approaching a toxic level of some element in the soil. For example, in mine tailings there may be quite toxic levels of some heavy metals such as zinc, copper, or lead. Normally little if any plant growth will occur in such material, but occasionally strains of plants have been found that will grow there at reasonable rates. The basic questions that cannot yet be answered to any extent concern the physiological mechanisms whereby these various species of plants, or strains of the same species, can tolerate these several sorts of mineral environment, whether these be saline, acid, or alkaline, or simply generally toxic. Nor of course is there any knowledge of the steps by which these plants adapt to such unfavourable conditions.

TRANSPORT OF NUTRIENTS IN THE PLANT

Nutrients absorbed from the soil have to be transported upwards through the stems and distributed to the various growing plant organs, and the products of photosynthesis have to be transported from the leaves and distributed through the entire plant. There are two distinct transport systems within a plant. Firstly there is the xylem system consisting essentially of open tubes of varying diameter. These carry the sap upwards from the root, through the stem and into the leaves and growing points. This is simply the movement of a fluid through an open system of pipes. At its many distal termini the water may evaporate leaving the dissolved nutrients in the organ to which they have been transported. The xylem characteristically transports upwards the nutrients absorbed by the roots and distributes them through the plant. The second transport system in the plant, the phloem system, consists essentially of a series of living cells ranging through the plant, usually alongside and parallel to the xylem. The way in which materials move through the phloem cells is not at all clear, but it too, is a most efficient transport system. Movement of materials in the phloem is a movement from one living cell to another, not just a movement through open tubes. Characteristically the phloem transports soluble carbohydrates away from the leaves to other parts of the plant, but this system also transports inorganic nutrients, e.g., phosphate or potassium, back from the leaves. There would thus appear to be the capacity for the circulation of such nutrients from one part of the plant to another.

This rather simple view of transport of nutrients within the plant has had to be modified in recent years in several important ways:

1. The classic view of transport of mineral nutrients in the xylem is that the nutrients are absorbed by the roots, transported across the root tissues, and enter the xylem vessels, up which they are to be transported quite passively. Techniques have been devised whereby one can extract the liquid contents of the xylem from a woody shoot in the laboratory. It is then possible to examine directly the nature and amount of materials present in the xylem stream. Most detailed observations have been made on the level and forms of the element nitrogen in xylem sap. In many woody species the level of nitrate, the form in which nitrogen is generally absorbed from the soil, is very low, most of the nitrogen being present as organic compounds. In the xylem sap of apple trees, for example, all of the nitrogen appears to be present as various amino acids and amides. The actual level of nitrogen present

varies greatly, depending on the time of year—from about 5ppm in dormant trees to about 200ppm in the spring. Regardless of this level, however, about 80–90 percent of the nitrogen is present in two compounds—aspartic acid and its amide, asparagine—with other amino acids present at lower levels. By changing the level of nitrogen fertilisation the level of nitrogen in the sap can be changed, but the pattern and proportionality of the nitrogen compounds changes little.

It thus appears that, in some plants at least, the xylem is not simply the carrier of nutrient elements as they are taken up from the soil. With the element nitrogen considerable metabolism can occur before the nutrients appear in the xylem. As the pattern of compounds for any species appears to be quite characteristic, quite specific synthetic processes have occurred soon after absorption of this element.

2. Examination of materials moving in the phloem cannot be attempted so directly, as, with most species, it is not possible to obtain samples of phloem sap. With a few species it is possible to collect a few drops of material exuded by the phloem tissues when a stem is cut, but the quantities available are small and examination correspondingly difficult. We do know from other evidence, however, that the carbohydrate most commonly transported in the phloem is sucrose—not glucose or fructose, which are the sugars most commonly found in leaf tissues. Sucrose is a most suitable transport compound because the cells of the phloem apparently do not contain the enzyme invertase, which hydrolyses sucrose. Thus sucrose moves through these tissues essentially as a protected compound. If glucose or fructose were to move in the phloem it is likely that they would be broken down by enzymes present in the phloem. It is also of interest that in a few species other carbohydrates move in the phloem in addition to sucrose, e.g., in several species of Rosaceae the sugar alcohol, sorbitol, moves in the phloem.

3. Some of the mineral elements also are transported in the phloem, e.g., some of the nitrogen that has been supplied to a given leaf can move out as that leaf ages and can travel to some of the younger developing leaves. This phenomenon of redistribution is known to occur regularly with most elements, and it is in the phloem tissues through which such redistribution occurs. Some idea of the extent of this redistribution can be gained at the end of the season in a deciduous plant. As the leaves age in autumn there is a considerable movement of nutrients from the leaves back into the perennial parts of a plant, and the extent to which this occurs can be measured. In the apple tree, for instance, it is known that something like 50 percent of the leaf nitrogen moves back; the amount of phosphorus is less but it is still something like 30 percent, and so on. This movement occurs at all times—not only at the end of the growing season. One element, however, behaves differently in this regard. Calcium apparently does not move back out of the leaves at the end of the growing season; neither does it appear to be redistributed from foliage once it is supplied there in the xylem stream. Such observations have led to the idea that calcium generally is immobile in the phloem of plants. It has been suggested that phloem tissues may contain a high proportion of oxalic acid, and that any calcium would simply be precipitated as calcium oxalate crystals. These crystals have been observed in phloem cells of some species, but it is difficult to believe that this is the only reason for the immobility of calcium in phloem tissues.

What are the implications of this phenomenon of calcium immobility for plant growth and development? Let us consider for a moment a developing plant organ, e.g., a developing fruit. Any such developing organ will receive part of the ascending sap stream and the nutrients contained therein. The proportion of these nutrients will be determined by the supply of each element from the root system. In addition to these nutrients contained in the xylem stream, developing fruits will also receive considerable nutrients through the phloem. These will consist of products of photosynthesis from the leaves and also some mineral nutrients passed on from the leaves. If it is correct that calcium is not mobile in the phloem, then developing fruits should not receive any of this element by this second route. In other words the proportion of calcium relative to other elements in developing fruits should be less than in the leaves. If we follow the accumulation of different elements in a growing fruit it can certainly be seen that the rate of build-up of calcium is much slower than the build-up of other elements. Perhaps, however, the most striking way of illustrating this is to compare the ratios of the concentration of elements in leaves to their concentration in fruits at different times during the growing season. With some elements such as nitrogen or potassium the ratio does not alter much during the season. The proportion of calcium, however, steadily increases in the leaves relative to the fruits. This supports the idea that the only supply of calcium reaching a developing fruit is that coming in the xylem stream. In other words, fruits on an apple tree are probably nearer calcium deficiency than are the leaves. It is possible that an actual deficiency of calcium may exist in certain organs on a plant that itself may be adequately supplied with calcium.

Is this a purely theoretical consideration or does it have any practical relevance? It is now believed that this situation occurs in certain crops to an important economic extent. For many years apples have been known to suffer from a disorder known as bitter pit. This consists of disfiguring pits that develop in the flesh of fruits as they approach maturity on the tree or after picking while in storage. Some years ago it was found out, more or less by accident, that trees sprayed with a solution of calcium nitrate produced fruit with much less pitting. The reasons for this effect are now, I think, apparent, and it would seem clearly that the affected fruits are suffering from a localised deficiency of calcium even though leaves on the same trees may have an abundance. We are now also beginning to appreciate that certain disorders in other developing fruits, such as tomatoes, may also be due to a localised shortage of calcium.

4. The fourth aspect of transport I would like to mention is the overall control of nutrient transport in a growing plant. What controls the movement of nutrients from one plant part to another? Why, for example, do nutrients, move from older to younger leaves? Plant physiologists have long recognised the existence of what they have called " physiological sinks "—these being simply regions or organs to which nutrients are apparently attracted. For example such developing organs as a growing fruit, or a swelling pea pod, or a developing potato tuber are all recognised as strong " sinks " to which nutrients move preferentially from other organs such as leaves, roots, etc.

Until recently we had no clue as to the mechanism of such attraction of nutrients. We now know that some of the plant hormones may be involved in this mobilisation of nutrients. For example it can be readily demonstrated with radioactive isotopes that the placing of a small amount of one of the cytokinins on a plant leaf results in substantial movement of nutrients to that region. I think it is likely that in the near future we will see much more research on this topic of control of nutrient movement in the plant.

RELATIONSHIPS BETWEEN THE VARIOUS PLANT ORGANS: PLANT HORMONES

It is quite obvious that there is specialisation of nutritional function among the various plant organs. Thus photosynthesis can proceed only where chlorophyll is present, and so the green parts of a plant must make enough sugar for the entire plant. The sugar needed by the roots, for example, must be transported there from the upper green parts. What do the roots contribute to the nutrition of the whole plant? Are they simply a mechanical device for anchoring the plant and for the passive transport of water and mineral nutrients from the soil? I have already discussed the fact that some of the nutrients passing up the plant have already been considerably metabolised in the roots. Other information on the inter-relations of root and shoot come from grafting experiments. One of the characteristics of the tobacco plant is the accumulation of an alkaloid, nicotine, in its leaves. A closely related species, the tomato, is normally completely free of this alkaloid. It is comparatively easy to graft a tobacco shoot on to a tomato root, and when this is done the shoot contains no new nicotine. When the reverse graft is carried out, that is, when a tomato shoot is grafted on to a tobacco root, we find that the tomato leaves begin to accumulate nicotine. It would thus appear that nicotine or some essential precursor of it is normally synthesised in the root system and travels to the foliage where it accumulates.

I have mentioned that the root of a plant is obviously dependent on the leaves for its supply of carbohydrate. We can get some idea of what else the root normally obtains from leaves by attempting to grow detached roots in culture. It is now possible to germinate seeds of several plant species under sterile conditions, to remove part of the young roots formed, and to grow these in suitable media. These usually contain certain vitamins as well as sugar and mineral salts. All the species of root so far grown in culture appear to require nicotinic acid, pyridoxin (vitamin B_6), and thiamine (vitamin B_1). Thus it would seem likely that, in intact plants, roots receive their supplies of these vitamins from the shoot. The roots of plants appear to require these vitamins because they are unable to synthesise them for themselves.

I have been discussing, so far, the nutritional relationships of one plant organ with another, but it is likely that the nutritional inter-relationships within an organ are more complex still. It is quite likely that one tissue will differ from another tissue in nutritional requirements even though both these tissues occur within the one plant organ. Just as plant roots can be grown in culture so is it now possible to grow various plant tissues in isolation. This technique of plant tissue culture is one in which there is currently considerable interest. In tissue culture a small homogeneous piece of plant tissue grows not into a definite organ such as a root, but simply into a mass of unorganised cells. Using this technique we can study the gross nutrient requirements of particular tissues. To grow any plant tissue in culture we must supply it with sugar, a number of mineral elements, and several vitamins. However, in most cases, it has also been necessary to add some natural extract. The additives most commonly used have been coconut milk and extracts of various seeds such as corn. Until recently these materials were simply added to tissue culture media without any knowledge of the active compounds contained in them. Coconut milk and corn seed extract are complex mixtures containing sugars, mineral salts, vitamins, etc., but gradually, one by one, these various components were shown not to account for the activity of the extract. The essential compounds in these extracts were, by inference, present in only small quantities. If coconut milk was not added to media, tissues would grow a little by cell expansion; but little or no cell division occurred unless coconut milk was present. Inclusion of coconut milk in the culture medium apparently stimulated cells to divide. These observations led to the concept of plant hormones responsible for the stimulation of cell division. These compounds were recognised and known as kinins or cytokinins, long before the nature of any of the individual compounds was determined.

Before saying anything further about cytokinins I would like to say a little in general about hormones in plants. The concept of hormones has of course come to us from animal physiology. The term " hormone " was applied first to special chemical compounds, produced in specific organs of the animal body, which moved from these organs and had a characteristic effect on tissues remote from the organ producing them. These substances, these hormones, had the task of co-ordinating many of the bodily functions: thus the thyroid gland produced certain characteristic thyroid hormones and the pancreas produced insulin; the adrenal glands and the pituitary glands each produced their own characteristic hormones. The vital role of hormones in the animal body is now well recognised. What is not so well recognised is the fact that the plant body also produces a number of compounds that function in an analogous manner. Yet 90 years ago Charles Darwin undoubtedly recognised the existence of such action: " We must, therefore, conclude than when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part, causing the latter to bend."

It is now generally believed that many phases of plant growth are subject to hormonal control. In 1926 the first type of plant hormone was recognised and was called an auxin. Auxins are characterised by their ability to cause growing stems to bend and to inhibit development of lateral buds on a plant stem. The compound responsible for auxin action was shown to be indole-acetic acid (Fig. 1), a relatively simple chemical compound, and work over the years since has amply confirmed that this compound and one or two related compounds are responsible for many effects in normal plant growth.

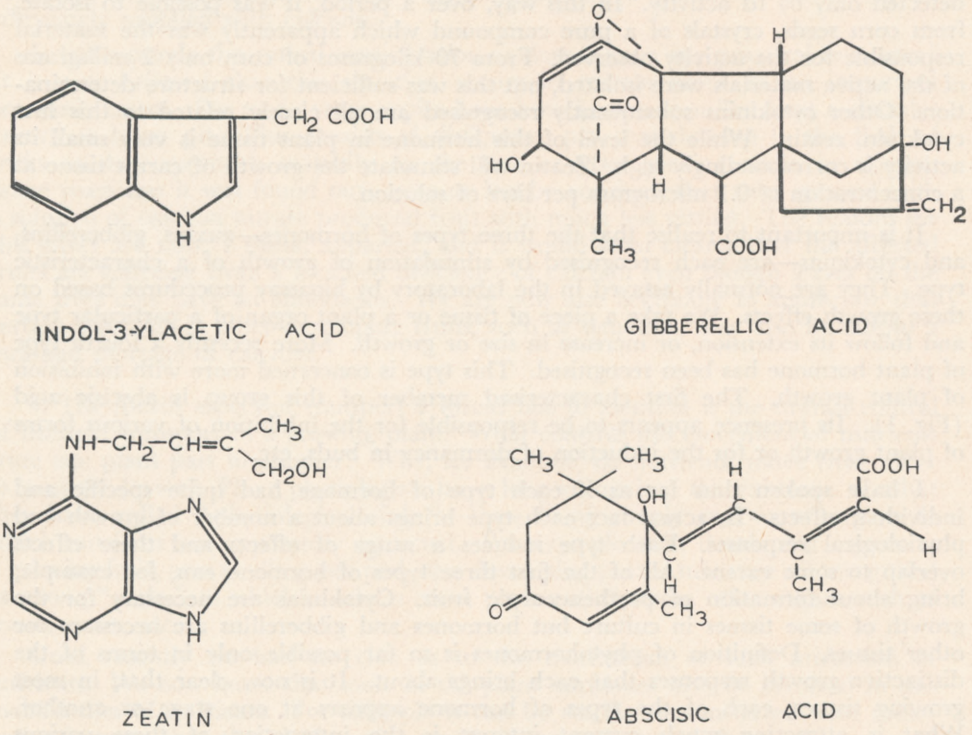

FIG. 1.—Formulae of naturally-occurring plant hormones.

The second group of plant hormones to be recognised were the gibberellins, and these were first noticed in a rather strange way. A disease of rice, common in Japan, is caused by a fungus, a species of *Gibberella*. A symptom of this fungus disease is that affected rice plants become taller and more spindly than their healthy neighbours. The Japanese had known this disease for some time, but it was not until the 1940's that Japanese workers were able to isolate from the fungus certain metabolic products which they called gibberellins. One of these compounds, gibberellic acid (Fig. 1) when applied to healthy plants caused similar extension of growth to that in the diseased rice plants. It was also found that gibberellic acid when applied to other plants caused them to grow taller than usual. This effect of a fungus product was interesting enough in itself, but what is now of more interest is that these gibberellin compounds are now known to be normal constituents of higher plant tissues. An extreme case is found in tall and dwarf peas, for example, or tall and dwarf corn, where the dwarf plants are deficient in some or all of the gibberellins but the tall varieties possess these compounds. We now know of more than 20 separate types of gibberellin compounds in higher plants, but they are all derivatives of the same basic chemical structure.

We now recognise a third group of plant hormones, the cytokinins. The first member of this group of compounds to be discovered was zeatin (Fig. 1), and as the work of isolation and determination of the structure of this compound was done in New Zealand it may be of particular interest. As discussed earlier the growth of plant tissues in tissue culture often depends on the addition of coconut milk or corn extract to the growth medium. The first isolation of one of these compounds was made from corn seed extract by a series of conventional chemical fractionation procedures. At each step one could detect the presence or otherwise of the hormone by performing a tissue culture growth experiment; the active compound could be detected only by its activity. In this way, over a period, it was possible to isolate, from corn seeds, crystals of a pure compound which apparently was the material responsible for the activity observed. From 70 kilograms of corn only 2 milligrams of the active materials were isolated, but this was sufficient for structure determination. Other cytokinins subsequently recognised are all closely related to this first cytokinin, zeatin. While the level of this hormone in plant tissue is very small its activity is correspondingly high. Zeatin will stimulate the growth of carrot tissue at a concentration of 0.1 microgram per litre of solution.

It is important to realise that the three types of hormones—auxins, gibberellins, and cytokinins—are each recognised by stimulation of growth of a characteristic type. They are normally assayed in the laboratory by bioassay procedures based on these growth effects. We take a piece of tissue or a plant organ of a particular type and follow its extension, or increase in size or growth. More recently a fourth type of plant hormone has been recognised. This type is concerned more with inhibition of plant growth. The first characterised member of this group is abscisic acid (Fig. 1). Its presence appears to be responsible for the inhibition of various forms of plant growth or for the induction of dormancy in buds, etc.

I have spoken thus far as if each type of hormone had quite specific and individual effects. In actual fact each type brings about a number of growth and physiological responses. Each type induces a range of effects and these effects overlap to some extent. All of the first three types of hormone can, for example, bring about formation of parthenocarpic fruit. Cytokinins are necessary for the growth of some tissues in culture but hormones and gibberellins are necessary for other tissues. Definition of phytohormones is so far possible only in terms of the distinctive growth responses that each brings about. It is now clear that, in most growing tissues, each of the types of hormone appears at one stage or another. What is attracting much current interest is the interaction of these various hormones in producing the various growth effects characteristic of each species of plant. It may be that one hormone in fact stimulates the development of another, which then brings about the observed action; in other cases there may be a genuine interaction between two hormones. This interaction may be synergistic or it may be antagonistic. The subtle interactions among the several types of plant hormone are giving us a new concept of how the whole plant is integrated, promising the development of a new science of " plant endocrinology ".

Little is known about the nature of the basic mode of action of each hormone. We now know something of the biological activities of each type but the mechanism of action at the molecular level is virtually unknown. Although these compounds in plants are analogous to hormones as understood in animal physiology, there are some contrasts. Animal hormones, for example, are relatively specific in action and most appear to have one principal function. The plant hormones, in contrast, do have some distinctive responses on which their specific bioassays are based, but they also have some apparently common biological activities which interact one with the other. The animal hormones are chemically quite distinct from the plant compounds. Most animal hormones are relatively large molecules, some of them small proteins. The plant compounds that have been recognised so far are relatively simple molecules.

REGULATION OF METABOLISM

One of the outstanding results of modern microbiology is the recognition that many micro-organisms, particularly bacteria, show a marked regulation of their own metabolism. It is now known that the metabolic pattern in microbial cells is not something that is fixed and constant. The pattern of enzymes present changes according to different situations. Some enzymes are present all the time; others are produced only when they are necessary. It is becoming increasingly apparent that in microbes there is a precise control of many aspects of metabolism. An outstanding recent achievement of molecular biology has been the working out of the nature of some of these processes in bacteria.

It is now of considerable interest to the plant physiologist to know whether similar phenomena occur in higher plant tissues. Here our knowledge is pretty meagre, but in recent years some information has come to light. In the nitrogen nutrition of higher plants we now have two instances of adaptive enzymes, that is, enzymes that are apparently not normally present but can be produced by the plants when needed.

The first example of these concerns the utilisation of nitrate as a source of nitrogen for plant growth. Plants are known to be able to use either nitrate-nitrogen or ammonium-nitrogen for growth. With either of these sources of nitrogen many plants will grow equally well. When nitrate is the source it is first necessary for the plant to reduce nitrate to ammonium before further metabolism can occur. The first step in this reduction of nitrate is effected by the enzyme nitrate reductase. It has now been shown clearly that this enzyme is not usually present in plants. For example, if we grow *Spirodela* with ammonium in the culture medium we find a negligible level of nitrate reductase in the plants. If, however, we grow the same plant with nitrate as the source of nitrogen a considerable level of this enzyme is found in the plant. The appearance of this enzyme when required is an example of the phenomenon of enzyme induction, and we say that the enzyme nitrate reductase is induced by the presence of nitrate.

A similar situation has also been shown to occur when we grow *Spirodela* with urea, an organic nitrogen compound, as sole source of nitrogen. In this case, the enzyme urease, which is normally not present in *Spirodela,* is found in quantities when the plants are growing with urea. The enzyme urease is responsible for the production of ammonia from the organic compound urea. Here apparently is a second example of an induced enzyme in higher plants. Interestingly enough the same two enzymes—nitrate reductase and urease—are now known to be induced enzymes in bacteria.

Let us look a little closer at the utilisation of ammonium or nitrate as sources of nitrogen for plant growth. If *Spirodela* is supplied with both ammonium and nitrate at the same time we find that ammonium is used first, and not until all the ammonium has been removed from the medium is there any considerable utilisation of nitrate. This means that, in *Spirodela,* the utilisation of nitrate must be controlled so that it cannot occur if ammonium ions are also available. The utilisation of nitrate can be divided into two stages; uptake of this material from the medium, followed by its reduction and assimilation to ammonium. The assimilation of nitrate to ammonium can be further divided into two stages: reduction of nitrate to nitrite, and subsequent reduction of nitrite to ammonium. Thus control of the utilisation of nitrate could be effected by control of the uptake of nitrate, the reduction of nitrate to nitrite, or the reduction of nitrite to ammonium. In practice it was found that, in *Spirodela,* only the first two processes were affected by the presence of ammonium. Uptake of nitrate was partially inhibited and reduction of nitrate was almost completely inhibited, whereas reduction of nitrite was not affected at all. Looking in more detail at the inhibition by ammonium of nitrate reduction we found, somewhat to our surprise, that *Spirodela* when supplied with both ammonium and nitrate contained high levels of nitrate reductase and high concentrations of nitrate, but yet did not reduce nitrate. Thus it seemed that the presence of ammonium was not affecting the production of the enzyme nitrate reductase but, in some way, was affecting the activity of this enzyme. Thus we have pinpointed, to some extent, the way in which the presence of ammonium regulates the utilisation of nitrate, but we have in no way determined the mechanism of this effect.

Changes in the activities and levels of enzymes are also known to occur in other situations in plants. For example, we have observed marked increases in the activities of certain enzymes as we allow plants to become deficient. If *Spirodela* plants that have been grown in a complete medium are transferred to a medium lacking in nitrogen, for example, then naturally enough those plants soon develop symptoms characteristic of nitrogen deficiency. However, these plants do not stop growth immediately when transferred to deficient media, and will often add considerably to their fresh weight before growth ceases. Even though the new medium may contain no nitrogen, such plants are apparently able to reutilise some of the nitrogen they absorbed when growing on the previous medium. Gradually, however, growth of these plants becomes inhibited through shortage of nitrogen. The reutilisation of reserve nitrogen apparently requires new and different enzymes, for, under these conditions, the plant produces a different array of enzymes. For example, we have found that nitrogen-deficient plants have a marked level of the enzyme urease. Similarly, plants deficient in phosphate have a higher level of the enzyme phosphatase. Such enzymes are apparently produced quite specifically in response to deficiencies of elements. It is natural to suppose that these enzymes are in fact used by the plant in the remobilisation of its reserve nutrients, but direct proof of this has not been obtained.

I have said enough, I think, to demonstrate that plant organisms do exhibit quite marked regulation of their own metabolism. This is one of those aspects of plant growth that have not yet got into the text books. When we read about plant metabolism we are inclined to get the impression that the reactions of metabolism are fixed and unchanging, whereas we now realise that many of these reactions can be inhibited or stimulated in various ways. The actual presence of a compound may induce the production of an enzyme necessary for the metabolism of that compound. The appearance of a specific deficiency may induce the appearance of enzymes that, to some extent at least, can compensate for the deficiency of that element. Again the presence of one ion, such as ammonium, may in some way affect the enzymes that normally metabolise other compounds, in this case nitrate. All of this points to the presence of a most complex and elaborate system of controls in metabolism. We still have much to learn about the actual biochemical pathways of metabolism, but it is also apparent that we have a vast extra amount to learn of the actual regulation of these pathways, their interactions, and how the whole pattern of growth is completely integrated.

CONCLUSION

Man has over the years attempted to improve his ability to grow more food in several different ways. First, he has continually selected the plants he grows—a process that has gone on largely unconsciously down the ages. If one type of wheat plant produced more growth than another or survived a bad year better, man was more likely to use seed from that plant for next season. If an apple tree produced larger or sweeter or more attractive fruit on one branch, man was more likely to select that branch for propagation. This century, since Mendelian inheritance was first understood, we have seen a great development of the science of plant breeding. Secondly, man has striven to improve the way he grows his plants. Over the years he has developed improved procedures for various aspects of their culture, their propagation, their manuring, and the prevention of losses from disease. All these techniques have been most profitable, resulting in the highly developed systems of agriculture that are possible today. Yet much of our improvement in methods of selecting and growing plants has been on a purely empirical basis. We have selected plants because they grew better or they yielded more, without in any way understanding why they grew better or why they yielded more; we have adopted new cultural practices, often without understanding the basis of their advantage.

During his history man has used many plants for food. Of these he has cultivated about 150 species to the extent that they are the commercial plants of today. Despite the improvements of agriculture in the past, pressure is increasing on man further to improve his food production. A recent report on plant sciences from the U.S. National Academy of Sciences (*The Plant Sciences Now and in the Coming Decade*, 167 pp.; 1966) considered that some of the most urgently needed research was on the biological characteristics of the world's principal food plants. Many workers feel that further improvement in plant production is going to be increasingly difficult, that it will be necessary to attempt to modify the growth of plants on something other than the largely empirical basis used up to now. For this to be possible we will need a much better understanding of plant metabolism and growth than we now possess. In this talk I have tried to give some idea of the state of our knowledge in one or two aspects of this subject.

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