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THE PRODUCTION OF HORMONES IN HIGHER PLANTS

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CONTENTS

I. INTRODUCTION

In order to understand growth and development it is necessary to analyse what are in fact coherent, continuous processes into a series of causes and effects. A great deal has been found out about the effects of hormones on the control of growth and differentiation in plants; this has provided some understanding of the causes of developmental changes. But the production of hormones is itself an effect which requires a causal explanation. Something is known about the regions of hormone production in the plant and the way in which hormones move from these regions. But very little is known about the cellular sites of hormone production or about the way in which hormone production is controlled. Physiological investigations of hormone production in whole organs or parts of organs do not in themselves shed much light on these problems. Biochemical investigations of hormone production by tissues or homogenates of tissues containing a mixture of cells provide information about the biochemistry of hormone production under experimental conditions, but they do not reveal which cells produce the hormones *in vivo*. Unless the cellular sites of hormone production are known, it is almost impossible to understand how hormone production within the plant is controlled at either a physiological or biochemical level.

The majority of this review is concerned with the production of auxin, about which a vast and confusing literature has grown up over the last forty-five years. The production of the more recently discovered hormones, the gibberellins, cytokinins, abscisic acid and ethylene, will be considered only briefly. The results of biochemical and physiological investigations of hormone production will be discussed in an attempt to obtain a clearer understanding of the cellular sites of hormone production and of the way in which hormone production is controlled. The major conclusion to which this discussion leads is that much of the hormone production in plants takes place as a consequence of cell death.

II. THE BIOCHEMISTRY OF AUXIN PRODUCTION

There is a great deal of evidence that the natural auxin of plants is indol-3yl-acetic acid (IAA) (Thimann, 1969). Members of almost every group of living organisms are known to be capable of producing IAA; it is formed by numerous species of bacteria (Roberts & Roberts, 1939; Stowe, 1955; Wichner & Libbert, 1968) and fungi (Gruen, 1959); it has been found in a variety of animals (Went & Thimann, 1937; Gordon & Buess, 1967) and is produced in developing chick embryos (Robinson & Woodside, 1937). Considerable quantities are excreted in human urine, which is one of the sources from which IAA was first isolated. The IAA in human urine is not simply derived from plant material in the diet, nor can more than a third of it be attributed to auxin production by the microflora of the gut: the majority is actually formed in the human body (Weissbach, King, Sjoersdma & Udenfriend, 1959). The rate of human auxin production, expressed in terms that permit comparison with auxin production in plants, is about $5–50 \times 10^{-12}$ g/mg/h. Coleoptile tips of *Avena* yield 50×10^{-12} g/mg/h (Went & Thimann, 1937). This comparison emphasizes that IAA production in plants should not be regarded as an isolated biochemical phenomenon.

1. *Animals, fungi and bacteria*

IAA is produced in animals as a consequence of tryptophan catabolism. It is by no means the major breakdown product: for example, the increased excretion of IAA which follows the oral administration of tryptophan to humans accounts for less than 0·1 % of the tryptophan metabolized (Weissbach *et al.*, 1959). The production of IAA can occur as a result of the transamination or the decarboxylation of tryptophan, but the former is the predominant route (Weissbach *et al.*, 1959; Gordon & Buess, 1967). These reactions yield indole pyruvic acid and tryptamine respectively (Fig. 1). Indole pyruvic acid can undergo decarboxylation to indole acetaldehyde, which is also formed by the action of amine oxidases on tryptamine. IAA is produced by the oxidation of indole acetaldehyde.

The production of IAA by bacteria and fungi which occurs when tryptophan is added to the culture medium follows similar pathways. Although tryptophan degradation via tryptamine has been conclusively demonstrated, most bacteria and fungi resemble animals in the greater importance of the transamination route (Libbert, Erdmann & Schiewer, 1970). Again, it is important to bear in mind that IAA is only one of several possible products of tryptophan catabolism; for example indole pyruvic acid is not only decarboxylated but can also be reduced to indole lactic acid; and indole acetaldehyde is not only oxidized to IAA but can also be reduced to tryptophol (Fig. 1). These substances have often been detected in microbial cultures which are degrading tryptophan (e.g. Kaper & Veldstra, 1958; Rigaud, 1970*a*, *b*). Only a small proportion of the tryptophan supplied is converted to IAA: in cultures of *Agrobacterium tumefaciens*, for example, the maximum efficiency is less than 2 % (Kaper & Veldstra, 1958).

The way in which indole pyruvic acid is converted to IAA in animals and microorganisms is not fully understood. Indole pyruvic acid is a rather unstable compound; in aqueous solutions, especially under alkaline conditions, it breaks down spontaneously to give a number of different products, including IAA, indole acetaldehyde and tryptophol (Bentley *et al.*, 1956; Kaper & Veldstra, 1958; Moore & Shaner, 1967). Against this background of spontaneous degradation it is difficult to obtain evidence for the participation of enzymes; and indeed there seems to be no reason to believe that indole pyruvic acid does not decarboxylate spontaneously *in vivo*, or that indole acetaldehyde is not spontaneously oxidized. Enzymic oxidation or reduction of indole acetaldehyde may also take place, and there is some evidence that both occur (Kaper & Veldstra, 1958; Rigaud, 1970*a*, *b*). The relative contributions of enzymic and non-enzymic processes are, however, difficult to assess. Tryptophan can be converted to IAA *in vitro* by incubating the amino acid with a purified transaminase from *Escherichia coli* (Gunsalus & Stamer, 1955) in the presence of α-keto glutarate (an amino group acceptor) and pyridoxal phosphate (the co-factor necessary for transamination) but in the absence of any other enzymes (A. R. Sheldrake, unpublished results). This is hardly surprising in view of the instability of indole pyruvic acid, but it emphasizes that, *in vivo*, IAA could be produced simply as a consequence of tryptophan transamination, although the yields might be increased by enzymes capable of decarboxylating indole

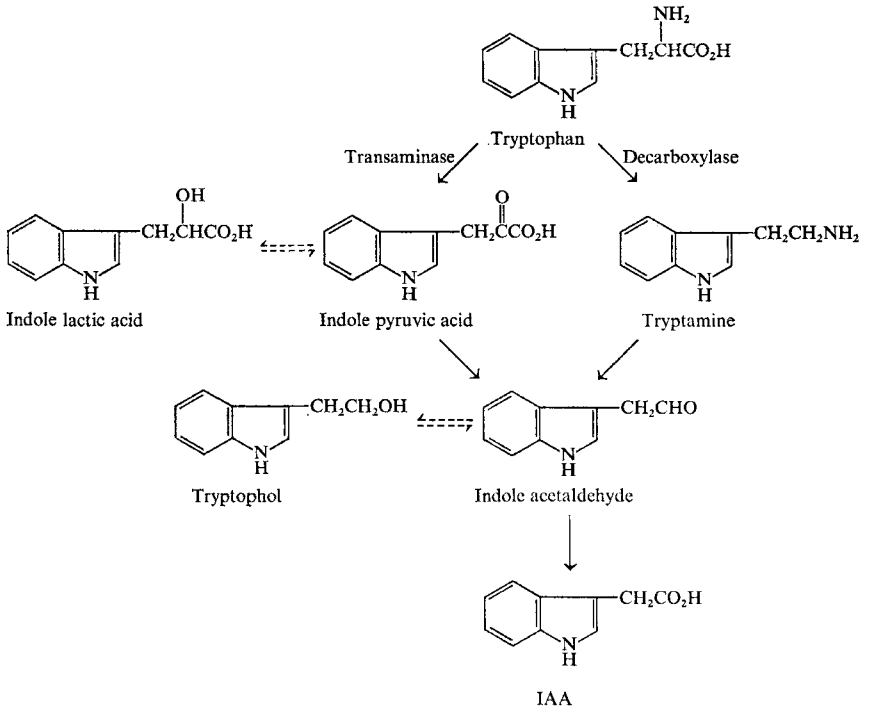

Fig. 1. Pathways of tryptophan degradation.

pyruvic acid and oxidizing indole acetaldehyde. Conversely, the yields might be diminished by enzymes which reduce these compounds to indole lactic acid and tryptophol.

IAA is not known to play a hormonal or indeed any other role in animals, fungi or bacteria. It is perhaps best regarded as a minor by-product of tryptophan catabolism, although for organisms pathogenic to plants it may be of importance in influencing the response of the plant to the pathogen.

2. *Higher plants*

Numerous investigations have shown that IAA is produced from tryptophan in plant tissues (for reviews see Gordon, 1961; Mahadevan, 1964; Libbert *et al.*, 1970; Wightman, 1973). Doubt was cast on the validity of some of the earlier results by Libbert *et al.* (1966) who showed that under some circumstances epiphytic bacteria could account for much of the auxin production by nonsterile plant tissues. The finding that sterile coleoptile sections of *Avena sativa* which grew in the presence of IAA did not elongate when tryptophan was supplied (Winter, 1966; Thimann & Growchowska, 1968) seemed to provide further evidence against tryptophan as a precursor of IAA. In order to explain their results, both Libbert *et al.* (1966) and Winter (1966) proposed hypothetical pathways of IAA synthesis from indole which did not involve tryptophan. Winter's conclusions were based on the assumption that coleoptile tissues normally synthesize IAA in the same way as other plant tissues. This

assumption is not valid, for it has long been known that coleoptile tips have an anomalous auxin economy which depends on a supply of auxin and/or 'inactive' auxins from the seed; there is no evidence to suggest that *de novo* IAA production occurs in coleoptiles (Section III, 1). Therefore the inability of sterile coleoptile tissues to produce auxin from tryptophan in sufficient quantities to stimulate growth does not support a general argument against the role of tryptophan as a precursor of IAA. In fact, sterile coleoptile tissues can produce small quantities of IAA from tryptophan (Libbert *et al.*, 1968; Libbert & Silhengst, 1970; Black & Hamilton, 1971). The ineffectiveness of exogenous tryptophan as an auxin precursor in sections of *Avena* coleoptiles appears to be due to its rapid incorporation into proteins, preventing any significant increase in the intracellular levels of free tryptophan (Black & Hamilton, 1971). There is good evidence that other sterile tissues can convert tryptophan to IAA (e.g. Kulescha, 1952; Libbert *et al.* 1968; Sherwin & Purves, 1969; Mitchell & Davies, 1972). Evidence against the hypothetical pathway of IAA synthesis from indole without tryptophan as an intermediate has been obtained by Erdmann & Schiewer (1971) and Black & Hamilton (1971). Libbert *et al.* (1970) have now concluded that IAA is, after all, formed from tryptophan in higher plants. Evidence for this view has continued to accumulate (e.g. Gibson, Schneider & Wightman, 1972; Wightman, 1973).

The predominant way in which plant tissues catabolize tryptophan is by transamination (Libbert *et al.*, 1970; Wightman, 1973). As in other organisms, the production of IAA occurs as a result of the breakdown of indole pyruvic acid. There is some evidence that this reaction can be catalysed enzymically, possibly by an oxidative decarboxylation analogous to the oxidative decarboxylation of pyruvic acid to acetylcoenzyme A. This evidence depends on the stimulatory effects of thiamine pyrophosphate (Gordon, 1961; Moore & Shaner, 1968) and lipoic acid (Gordon, 1961), which are co-factors in other oxidative decarboxylations. There is also evidence that indole acetaldehyde, which can be detected as an intermediate in the production of IAA from tryptophan by the use of radioactive tracers and/or trapping agents such as 2,4-dinitrophenyl hydrazine (Phelps & Sequira, 1967; Khalifah, 1967; Wightman & Cohen, 1968; Moore & Shaner, 1968; Gibson, Schneider & Wightman, 1972), may be oxidized enzymically. Aldehyde dehydrogenases capable of carrying out this reaction have been detected in a variety of plant tissues (Rajagopal, 1967; Wightman & Cohen, 1968; Gibson, Schneider & Wightman, 1972), although the presence of these enzymes does not in itself prove that they are normally involved in IAA production. It is at present almost impossible to assess the relative contributions of enzymic and spontaneous reactions in the formation of IAA from indole pyruvic acid *in vivo*.

In their recent review, Libbert *et al.* (1970) critically examined the evidence in favour of the formation of IAA from tryptophan via tryptamine and concluded that in higher plants the formation of tryptamine from tryptophan was unproven and unlikely. However, there seems to be no doubt that tryptamine can be detected in some, but not all, plant tissues (for references see Schneider, Gibson & Wightman, 1972). Several hypothetical pathways to account for its formation without the involvement of tryptophan have been proposed (Libbert *et al.*, 1970) but there is now persuasive evidence that some higher plant tissues do in fact contain enzymes capable of

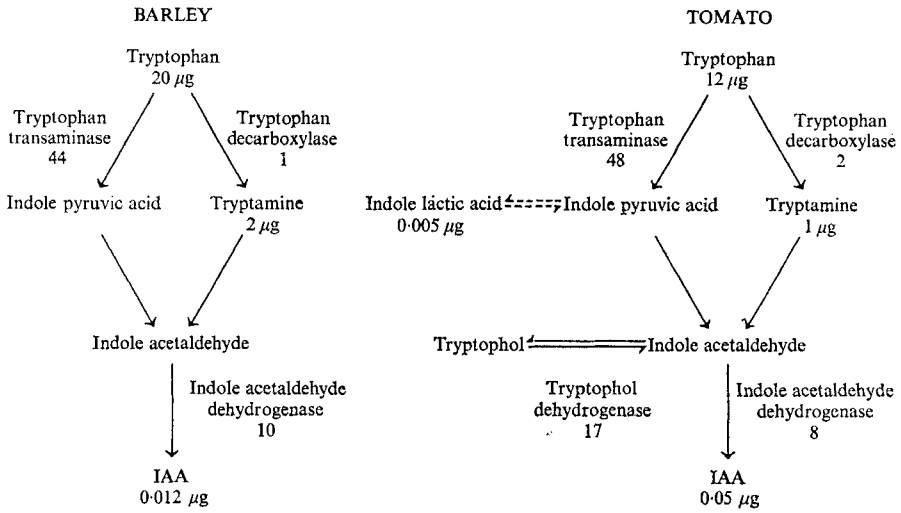

Fig. 2. Pathways of IAA formation. The numbers below the names of the indole compounds represent the concentrations in untreated tissue in μg/g fresh weight; numbers below the names of enzymes represent their rates of activity as μg product/g fresh weight/h. (Gibson, Schneider & Wightman, 1972).

converting tryptophan to tryptamine (Sherwin, 1970; Gibson, Schneider & Wightman, 1972; Gibson, Barrett & Wightman, 1972; Wightman, 1973). Some, but not all, plants contain amine oxidases which can convert tryptamine to indole acetaldehyde. The most intensive investigation of these enzymes has been carried out with pea (*Pisum sativum*) tissues (Mann, 1955; Clark & Mann, 1957) but unfortunately this is a species which does not contain tryptamine (Schneider *et al.*, 1972). Gibson, Schneider & Wightman (1972) have shown that in tomato (*Lycopersicon esculentum*) and barley (*Hordeum vulgare*) tissues, both of which normally contain tryptamine, tryptophan can be converted to tryptamine and this can in turn be converted to IAA. Enzymes capable of forming IAA from tryptophan via indole pyruvic acid are also present. These authors have estimated the relative activities of the enzymes involved in the degradation of tryptophan and also the naturally occurring amounts of tryptophan and its degradation products in these tissues. Their results (Fig. 2) indicate that the tryptamine pathway is relatively unimportant. Further results from the same laboratory have confirmed that in tomato shoots the primary pathway for the production of IAA from tryptophan is via indole pyruvic acid (Wightman, 1973).

A number of plants from a wide range of families have been found to contain 5-hydroxytryptamine, known to animal physiologists as serotonin (Schneider *et al.*, 1972). In some, for example in the stinging hairs of nettles (Collier & Chesher, 1956), it has a role in defence against animals; in others its function is unknown. In animals 5-hydroxytryptamine is formed by the decarboxylation of 5-hydroxytryptophan (Udenfriend, Titus, Weissbach & Peterson, 1956) but in plants it is probably formed by the hydroxylation of tryptamine (Gibson, Schneider & Wightman, 1972). Tomato tissues contain about five times more 5-hydroxytryptamine than tryptamine (Schneider *et al.*, 1972).

Tryptophan, in common with a number of other amino acids, can be degraded *in vitro* by reaction with phenols under alkaline conditions; the mechanism appears to involve an oxidative deamination by the quinones formed by the oxidation of phenols such as catechol (Gordon, 1961). The incubation of catechol with tryptophan at high pHs, and under less alkaline conditions in the presence of phenolase, leads to the production of small amounts of IAA (Gordon & Paleg, 1961; Gordon, 1961; Whitmore & Zahner, 1964; Wheeler & King, 1968). Gordon & Paleg (1961) suggested that this route of IAA production is probably of little significance *in vivo* because of the compartmentalization of the substrates in living cells, but they stressed its possible importance in macerated plant tissues and during extraction procedures. This point has also been emphasized by Whitmore & Zahner (1964).

Wightman (1973) has shown that when labelled phenylalanine is supplied to tomato shoots, a number of breakdown products including phenylpyruvic acid and phenylacetic acid are produced. The major pathway of phenylalanine degradation, like that of tryptophan, is by transamination; indeed the same transaminase is probably involved. Phenylacetic acid has weak auxin activity and occurs naturally in tomato shoots (Wightman, 1973).

3. *The biochemical control of auxin production*

Plant tissues convert tryptophan to auxin with efficiencies as low as, or lower than, those found in animals, bacteria and fungi. In short-term experiments, IAA rarely accounts for even as much as 0·1 % of the tryptophan supplied. Tryptophan is converted to auxin by auxin-requiring tissue cultures and can substitute for auxin as a growth substance if supplied at concentrations about a hundred times higher, implying an efficiency of conversion of about 1 % (Kulescha, 1952). None of the enzymes thought to be involved in the production of IAA from tryptophan have high specificities. For example, a purified 'tryptophan transaminase' from *Phaseolus aureus* has a higher activity with alanine, leucine, methionine, arginine, lysine, phenylalanine and tyrosine than with tryptophan (Treulson, 1972). A purified tryptophan decarboxylase from tomato shoots is much more specific for tryptophan, but this enzyme is of little importance in the production of IAA (Wightman, 1973). An amine oxidase from pea seedlings capable of oxidizing tryptamine to indole acetaldehyde also oxidizes a wide range of monoamines (Mann, 1955). And there is no evidence to suggest that enzymes which are able to oxidize indole acetaldehyde to IAA are specific for this substrate. The unspecific nature of these enzymes, the probability of spontaneous breakdown of intermediates such as indole pyruvic acid and the low efficiencies of IAA production from tryptophan suggest that in plants, as in other organisms, IAA is formed rather unspecifically as a by-product of tryptophan catabolism. It would be misleading to think of the reactions leading to the production of IAA as a biosynthetic pathway directly comparable to the efficient and specific enzyme pathways involved in intermediary metabolism or in most biosyntheses. A consequence of this view is that the control of IAA production from tryptophan is not likely to involve any very specific regulatory mechanisms at the enzymic level.

Several attempts to explain the control of IAA production in this way have been

made, but they are not persuasive. Rajagopal & Larsen (1972) purified an enzyme from non-sterile *Avena* coleoptile tissues which catalysed the conversion of indole acetaldehyde to IAA. They found that the best preparations under optimal conditions had a turn-over number of six molecules of indole acetaldehyde/min/enzyme molecule and suggested that this extremely sluggish activity might be of decisive importance in the control of IAA biogenesis. This conclusion seems improbable if only for the reason that coleoptile tissues do not normally synthesize IAA. For similar reasons, the findings that gibberellic acid enhances the growth of non-sterile coleoptile sections in the presence of tryptophan (Sastry & Muir, 1965) and that gibberellic acid has a promotive effect on the decarboxylation of tryptophan by homogenates of coleoptile tissues (Valdovinos & Sastry, 1968) do not support these authors' conclusions that auxin biosynthesis is regulated by gibberellic acid. These attempts to demonstrate a direct effect of gibberellic acid on auxin biosynthesis stemmed from observations that auxin production was enhanced in organs stimulated to develop by the application of gibberellins. For example, tomatoes stimulated to develop parthenocarpically by gibberellic acid produce auxin (Sastry & Muir, 1963). But so do tomatoes developing parthenocarpically after other chemical treatments (Section III, 7), indicating that auxin is probably produced as a consequence of fruit development. Similarly, the enhanced auxin production by dwarf peas and *Helianthus* plants stimulated to grow by gibberellins (Kurashai & Muir, 1962) and in rosette plants of *Centaurea* stimulated to bolt by gibberellins (Kurashai & Muir, 1963) seems likely to be a consequence of the developmental changes brought about over a period of days rather than as a direct effect of gibberellic acid on auxin biosynthesis. It is therefore difficult to evaluate the significance of the finding that enzyme preparations from pea tissues pretreated with gibberellic acid produced more 'ether-insoluble auxin' from tryptophan than controls (Muir, 1964). Valdovinos & Ernest (1966) found that homogenates of plant tissues which had been pretreated with gibberellic acid in a detergent solution released more $^{14}CO_2$ from [1-^{14}C]tryptophan than controls; but even if this effect was due to the gibberellic acid rather than the detergent, its relevance to the control of IAA production is far from clear.

The widespread occurrence of enzymes capable of catabolizing tryptophan with the consequent production of IAA in animals, fungi, bacteria and higher plants suggests that the major factor controlling the production of IAA is the availability of tryptophan; many cells and tissues do not produce IAA in significant quantities unless exogenous tryptophan is supplied. But all cells capable of protein synthesis must contain a pool of free tryptophan. Therefore it is probable that these endogenous levels of tryptophan are normally too low for tryptophan breakdown to occur. This suggests that the affinities of the tryphophan-activating enzymes (responsible for charging specific transfer RNAs) are higher than the affinities of tryptophan-degrading enzymes for tryptophan. The few available data on Michaelis constants (i.e. the substrate concentration at which an enzyme is half saturated) support this view: amino-acid-activating enzymes generally have K_ms between 1×10^{-6} and 1×10^{-4} M (Novelli, 1967), while the tryptophan transaminase of *Phaseolus aureus* has a K_m of $1{\cdot}7 \times 10^{-3}$ M according to Gamborg & Wetter (1963) and $3{\cdot}3 \times 10^{-4}$ M according to Treulson (1972).

Tryptophan transaminase and tryptophan decarboxylase from tomato shoots have K_ms of 5×10^{-3} M and 3×10^{-3} M respectively (Gibson, Barrett & Wightman, 1972).

The pathways of amino-acid biosynthesis have been studied most extensively in micro-organisms. In general, they are controlled by feed-back inhibitions whereby the amino acid which is the product of the pathway inhibits an enzyme, or enzymes, involved in its production, often at the beginning of the pathway; this inhibition occurs when the concentration rises above a certain level (Umbarger, 1969). Similar control mechanisms have been found to regulate amino-acid biosynthesis in higher plants (Miflin & Cave, 1972; Miflin, 1973). In micro-organisms, tryptophan biosynthesis is regulated by the inhibition of anthranilate synthetase, an enzyme at the beginning of the pathway, by tryptophan (Umbarger, 1969). There is evidence that tryptophan is synthesized by a similar pathway in higher plants (Delmer & Mills, 1968) and it seems reasonable to assume that the control mechanism may be similar. Tryptophan biosynthesis must be regulated in such a way that levels of tryptophan sufficient for protein synthesis are maintained, but these concentrations must be too low for tryptophan degradation to occur under most circumstances; otherwise all plant tissues would produce auxin all the time.

It is conceivable that auxin production could be regulated by a change in this control mechanism such that higher concentrations of tryptophan were synthesized. But a simpler way in which the tryptophan levels could be elevated is by the degradation of proteins. The free amino-acid pools in plant tissues represent only a small proportion, often less than 5%, of the total amino acids which can be released by the hydrolysis of the proteins (Allsop, 1948; McKee, 1958). In living cells, where there is a steady turnover of proteins, proteolysis presumably contributes to the steady-state pools of free amino acids. But when net protein degradation occurs, for example in senescent leaves, the levels of free amino acids are elevated considerably (Chibnall, 1939; McKee, 1958).

The amounts of free tryptophan in plant tissues usually lie in the range of 10–50 μg/g fresh weight (Schneider *et al.*, 1972). Higher amounts are found in shoot tips, young leaves, senescent cotyledons (Nitsch & Wetmore, 1952) and senescent leaves (Kim & Rohringer, 1969), all of which are sites of auxin production (Section II). These measurements give no information about the cellular distribution or intracellular localization of tryptophan. It is therefore impossible to deduce the intracellular concentrations.

The elevated tryptophan levels in senescent leaves and in the cotyledons of seedlings can be explained as a result of protein degradation. The elevated amounts of tryptophan per unit weight in shoot tips and developing leaves could in part reflect the higher ratio of cytoplasm to cell wall material in young tissues, and in part be explained by the net protein breakdown which occurs during vascular differentiation: xylem cells and most fibres undergo a complete autolysis as they differentiate, and partial autolysis occurs in differentiating sieve tubes.

Many D-amino acids are converted by plant tissues to their *N*-malonyl derivatives (Rosa & Neish, 1968) by what appears to be a detoxification mechanism. An analogous conversion of D-amino acids to their *N*-acetyl derivatives occurs in yeast (Zenk & Schmitt, 1965). Malonyl-D-amino-acid conjugates occur naturally in a wide range of

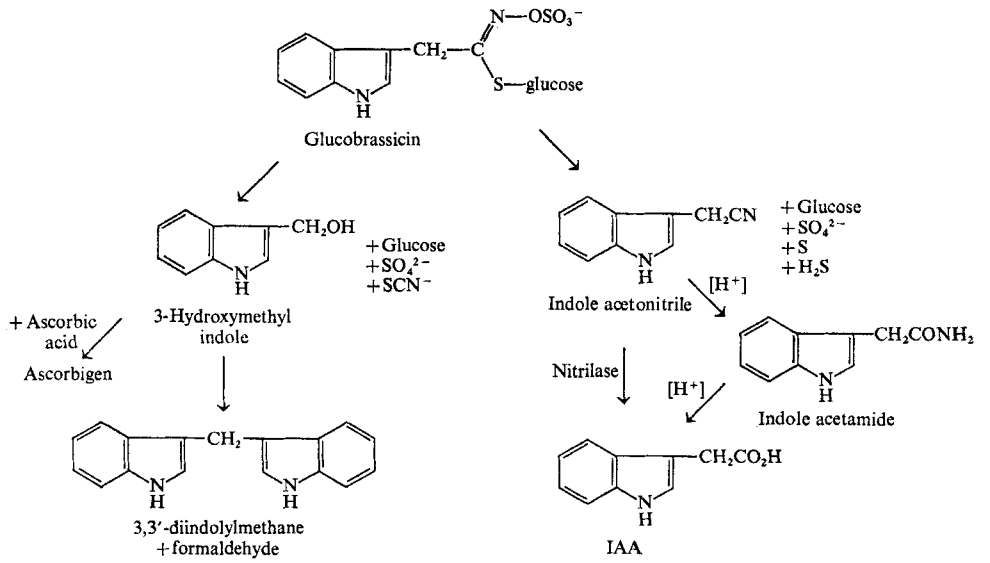

Fig. 3. The degradation of glucobrassicin (after Gmelin, 1964).

plants (Rosa & Neish, 1968) and malonyl-D-tryptophan has been detected in a number of vegetative tissues and fruits (Good & Andreae, 1957; von Raussendorf-Bargen, 1962; Zenk & Scherf, 1963). Apple (*Malus pumila*) fruits contain about 0·2 μg/g fresh weight (Zenk & Scherf, 1963) and tomato shoots about 0·5 μg/g fresh weight (Good & Andreae, 1957). This compound appears to be quite stable in plant tissues (Good & Andreae, 1957). It is possible that in autolysing tissues it could be hydrolysed, releasing free D-tryptophan which can be broken down by some plant tissues to IAA (e.g. Kim & Rohringer, 1969). However, the amounts of malonyl-D-tryptophan are so small compared with the amounts of L-tryptophan in plant tissues that it is unlikely to be of any significance for auxin production.

4. *Other indole compounds*

A number of plants have been used for centuries as a source of indigo dye, which is formed by the oxidation and polymerization of indoxyl. *Indigofera* and *Polygonum tinctorium* contain indoxyl-β-D-glucoside and woad (*Isatis tinctoria*) contains indole-β-D-5-ketoglucuronic acid (Stowe, Vendrell & Epstein, 1968). Indoxyl is released from these compounds by hydrolysis. Little is known about the indole metabolism of the plants which produce these compounds, but there is no reason to suppose that they are precursors of auxin or play any part in growth control.

A variety of mustard oil glucosides, or glucosinolates, are found in the Cruciferae, Resedaceae, Tropaeolaceae and certain other families. They appear to be stable *in vivo*, but when tissues are crushed or otherwise disrupted they rapidly break down under the influence of the enzyme myrosinase, which is probably confined to special cells (Virtanen, 1965). This reaction is of culinary importance in the preparation of mustard and is also responsible for the release of the compounds which give plants

such as cress (*Lepidium sativum*) and *Tropaeolum* their characteristic flavours (Virtanen, 1965). *Brassica* and a number of other genera contain the indole-glucosinolates glucobrassicin and neoglucobrassicin. These compounds are present in both roots and shoots in quantities which can account for up to 3% of the dry weight (Gmelin, 1964; Virtanen, 1965; Elliott & Stowe, 1971). Either spontaneously under acid or alkaline conditions, or as a result of the action of myrosinase, glucobrassicin breaks down to form a variety of indole compounds (Fig. 3), the nature of the products being determined by the conditions of the reaction (Gmelin, 1964; Virtanen, 1965). One of the breakdown products, 3-hydroxymethyl indole, dimerizes with the elimination of formaldehyde, but if ascorbic acid is present reacts with it spontaneously to form ascorbigen. The pH optimum of this reaction is 5 (Schraudolf & Weber, 1969). Indole acetonitrile is produced enzymically by myrosinase, but only if the pH is below 5·2 (Schraudolf & Weber, 1969). Reports of the occurrence of indole acetonitrile and ascorbigen in tissues of *Brassica* were made before the discovery of glucobrassicin, but these compounds are now known to have arisen as extraction artifacts. Little or none can be detected after extraction procedures that minimize the breakdown of glucobrassicin (Kutáček & Procházka, 1964; Schraudolf & Bergmann, 1965).

Indole acetonitrile forms indole acetamide spontaneously under acidic conditions; indole acetamide can be hydrolysed to IAA. The conversion of indole acetonitrile to IAA is also effected by the enzyme nitrilase which is present in some, but not all, plant tissues (Thimann, 1953). Indole acetonitrile can, if supplied to tissues which contain nitrilase, act as an auxin precursor; but there is no evidence that it has any such role *in vivo*. The hypothesis that indole acetonitrile was involved as an intermediate in auxin production (e.g. Wightman, 1962) depended on the belief that it was of natural occurrence in species now known to contain glucobrassicin.

The functions of glucobrassicin and neo-glucobrassicin, like those of many other secondary plant products, are unknown. There is no evidence that under normal conditions they act as auxin precursors or are involved in the regulation of growth. But since they break down when tissues are crushed or damaged, it is possible that auxin could be produced from glucobrassicin under pathological conditions. Gmelin (1964) has suggested that a process of this sort might explain the existence in the cabbage family of growth abnormalities similar to those induced by exogenous auxin. Some support for this view comes from the finding that in club-roots of *Brassica*, caused by the pathogen *Plasmodiophera brassicae*, indole acetonitrile is present, whereas it is not detectable in uninfected roots (Tamura, Nomoto & Nagao, 1972).

5. *Bound auxin*

It has long been known that animals possess a variety of detoxifying mechanisms which involve the formation of amino-acid or sugar conjugates. Benzoic acid, for example, is conjugated with glycine to form benzoyl glycine (hippuric acid) which is then excreted in the urine. In humans and chimpanzees glutamine complexes are also formed (Thierfelder & Sherwin, 1914). A wide range of compounds including phenylacetic acid and various substituted benzoic acids are converted to glycosides of

D-glucuronic acid (Teague, 1954). IAA complexes from which IAA can be released by acid hydrolysis are present in human urine (Weissbach *et al.*, 1959) and in Hartnup disease, which is associated with a greatly elevated excretion of IAA, up to 150 mg/day of IAA-glutamine is found in the urine (Jepson, 1956).

Similar detoxification mechanisms occur in plants. The administration of unphysiologically large amounts of IAA results in the formation of IAA complexes whose nature depends on the species of plant. In some only IAA-L-aspartic acid is formed, in others only IAA-β-D-glucose; but in most species both these compounds are produced (Zenk, 1964). Indole acetamide, which was previously thought to be formed from IAA as a detoxification product (Andreae & Good, 1957) is now known to have arisen as an artifact by the breakdown of IAA-glucose during chromatography in ammoniacal solvents (Zenk, 1961). A number of synthetic auxins are converted by the same mechanisms to aspartic-acid and/or glucose derivatives (Andreae & Good, 1957; Zenk, 1962; Südi, 1964).

IAA-glucose can be detected soon after the application of exogenous auxin to leaf discs of *Hypericum hircinum*, but IAA-aspartate begins to appear only after a lag period of about 2 h at 25 °C (Zenk, 1964). The levels of IAA-glucose decline as IAA-aspartate is formed. The lag period preceding the formation of IAA-aspartate can be eliminated by pretreating tissues with IAA or synthetic auxins such as naphthalene acetic acid (Andreae & Ysselstein, 1960; Südi, 1964). This effect is prevented by inhibitors of protein synthesis (Venis, 1970), suggesting that it is due to enzyme induction.

One day after the administration of IAA to *Hypericum* tissues, the majority of the IAA taken up by the tissue is found to have been converted to IAA aspartate (Zenk, 1964). A small proportion of the IAA remains in a free form at a steady-state level of about 6 μg/g fresh weight (Zenk, 1964). This is well above the normal levels of IAA encountered in vegetative tissues, which usually lie in the range of 0·005–0·050 μg/g fresh weight (for references see Schneider *et al.*, 1972). The fact that neither IAA-aspartate nor the enzyme system which forms it are normally detectable in untreated vegetative tissues indicates that the endogenous auxin levels are too low for this conjugation of auxin to occur under physiological conditions. There are, however, times and situations in plants where relatively large quantities of auxin are produced, for example during seed development, and the conjugation of auxin may be of considerable physiological importance under these circumstances. A number of dicotyledonous seeds and fruits have been shown to contain IAA-aspartate (Klämbt, 1960; von Raussendorf-Bargen, 1962; Zenk, 1964) and it has been known for many years that large quantities of bound auxin are produced during the development of cereal grains.

In developing rye seeds there is at first a large increase in the amount of free auxin followed by a decline associated with the formation of substances from which auxin can be released by mild alkaline hydrolysis (Hatcher & Gregory, 1941; Hatcher, 1943), suggesting that an ester linkage is involved. Developing maize kernels contain both free and bound auxin; the levels of free auxin decline as the seed matures (Avery, Berger & Shalucha, 1942; Hemberg, 1958; Hamilton, Bandurski & Grigsby, 1961).

About half the bound auxin of maize seeds consists of low molecular weight esters which have been identified as IAA-inositol complexes (Ueda & Bandurski, 1969). Both lipid-soluble and high molecular weight esters are also present (Takano, Bandurski & Kivilaan, 1967; Ueda & Bandurski, 1969). Hemberg (1955) suggested that the bound auxin formed during seed development serves as a source of free auxin in the germinating seed. It is on the auxin released by the hydrolysis of these auxin 'precursors' that the vicarious auxin economy of the coleoptile tip depends (Section III, 1).

Auxin is not the only hormone which is converted to a bound form. Glucosides of abscisic acid (Milborrow, 1970) and gibberellic acids occur naturally in plants. The latter are formed during seed development from free gibberellic acids; these, like auxin, are liberated by hydrolysis from their bound forms in germinating seeds and embryos (Barendse, Kende & Lang, 1968; Barendse, 1971; Dale, 1969).

For many years the possible existence of auxin-protein complexes has been shrouded in confusion and controversy. The release of auxin from proteins by alkaline hydrolysis or by proteolytic enzymes has often been taken as evidence for the existence of protein-bound auxin. But results of this type can also be explained by the release of tryptophan, followed by the conversion of tryptophan to IAA. This conversion takes place spontaneously under alkaline conditions (Gordon & Wildman, 1943). Auxin is produced by the alkaline digestion of casein, but not of gelatine which contains negligible amounts of tryptophan (Gordon & Wildman, 1943). Wildman & Bonner (1947) found that auxin was released by the hydrolysis of spinach leaf protein in quantities greater than they estimated would be produced by the spontaneous conversion of tryptophan; they concluded that an auxin-protein complex was present. However, Schocken (1949) showed that Wildman & Bonner's assumptions about the rate of spontaneous production of auxin from tryptophan were wrong. He re-investigated the production of auxin from the spinach 'auxin-protein' and found that similar yields of auxin were obtained by the hydrolysis of a number of animal proteins; the amount of auxin produced was roughly proportional to the tryptophan content of the protein. Wildman & Bonner's evidence in favour of the 'auxin-protein' also depended on enzymic digestions, but the incubation conditions (48 h at 37 °C under non-sterile conditions) make it probable that much of the auxin detected was formed by micro-organisms.

Winter & Thimann (1966) claimed that part of the IAA which is immobilized in coleoptile tissues during polar auxin transport is in the form of an auxin-protein complex. The majority of labelled IAA present in the tissues at the end of the transport period was found in the sediment when ground-up coleoptile tissue was centrifuged. Radioactivity was released from this fraction by treatment with proteolytic enzymes or urea. However, the binding of IAA was very labile since IAA could be recovered by extraction with ether. These authors were apparently unaware of the ability of IAA to partition into lipid membranes in a pH-dependent manner (Hertel, Thomson & Russo, 1972) much in the same way that IAA partitions into non-polar solvents at pHs below about 5. Their experiments involved either unbuffered solutions, or a comparison of various treatments in solutions buffered at different pHs.

Their results may therefore be explicable in terms of a pH-dependent association of IAA with membranes.

Siegel & Galston (1953) claimed to have demonstrated the formation of an IAA-protein complex *in vitro* by plant homogenates. The binding of IAA to protein was stimulated by nucleotides. These results are now known to have been due to an artifact caused by the use of tri-chloro-acetic acid as a protein precipitant (Zenk, 1964). Evidence for the formation of an IAA-transfer RNA complex (Bendana *et al.*, 1965) has also been refuted (Davies, 1971). In a recent publication from the same laboratory the formation of IAA-polysaccharide complexes has been postulated (Davies & Galston, 1971), but the evidence for their existence is indirect.

Zenk (1964) has provided the only convincing evidence for the existence of an IAA-protein complex. A small proportion of labelled IAA supplied to pea epicotyl tissues was found to be bound to a protein fraction from which it could not be removed by treatment with acetone, dialysis or by exchange with unlabelled IAA. These results suggest that the IAA was chemically bound to the protein. It is not known whether this IAA-protein complex has any physiological significance, or indeed whether it is formed at all under natural conditions.

In extraction procedures, 'free' auxin is taken to be the auxin obtained after short periods in the cold, whereas the additional auxin obtained after longer periods of extraction has been considered to be due to the release of free auxin from a 'bound' form (Bentley, 1961). This definition of bound auxin is confusing. In some cases, for example in the extraction of cereal seeds (e.g. Hemberg, 1955) and coleoptiles (Wildman & Bonner, 1948) this bound auxin represents genuine IAA complexes such as the IAA-inositol compounds. The free auxin which can be liberated from the 'bound auxin' of *Brassica* tissues (e.g. Avery, Berger & White, 1945) is probably formed as a consequence of the breakdown of glucobrassicin. In other cases the 'bound auxin' represents IAA produced from tryptophan during extraction. The addition of proteolytic enzymes to tissues leads to larger yields of IAA during extraction (Skoog & Thimann, 1940; Thimann, Skoog & Byer, 1942) probably because more tryptophan is released from proteins. No auxin production occurs when boiled tissues are subjected to prolonged extraction with ether (Thimann & Skoog, 1940).

6. *The production of auxin by autolysing tissues*

The continued production of auxin by plant tissues as they autolyse during extraction with ether is probably due to the continued release of free tryptophan from proteins and its subsequent degradation. But it is not only during ether extractions that autolysis results in auxin production. Yeast, plant and rat liver tissues produce auxin as they autolyse *in vitro* (Sheldrake & Northcote, 1968*a*). Increases in the amount of auxin of up to a hundredfold can occur within 24 h.

The production of auxin by autolysing tissues *in vitro* suggests that autolysing tissues within the plant might also produce auxin. But it would be almost impossible to duplicate in the test tube the complex sequence of changes that occur during the autolysis of a differentiating xylem cell, to take only one example. The pH of the different cellular compartments may change as differentiation proceeds and as the

intracellular membranes break down; the concentrations of tryptophan would be expected to change as it is released from intracellular compartments (e.g. plastids) and the amount produced by proteolysis would be affected by the changing compartmentalization of proteolytic enzymes and the changing pH in which they are operating. These factors would also affect enzymes which break down tryptophan. It is also probable that some of the substances released as the cell autolyses would be metabolized by adjacent living cells.

The techniques of biochemistry are at present too crude to investigate in any detail changes of this type as they occur *in vivo*. From a biochemical point of view it seems probable that autolysing cells would produce auxin; but biochemical investigations alone cannot reveal the extent and significance of auxin production by such cells within the intact plant. The problem must now be considered at the physiological and anatomical levels.

III. SITES OF AUXIN PRODUCTION IN THE PLANT

With the exception of senescent leaves and coleoptile tips, practically all the sites of auxin production in the plant are in regions of meristematic activity. This correlation between auxin production and meristematic activity has led to the widely, if implicitly, accepted hypothesis that auxin is produced by meristematic cells. A very different hypothesis of auxin production has recently been proposed, according to which auxin is produced as a consequence of cell death (Sheldrake & Northcote, 1968*a*, *b*). The production of auxin by autolysing tissues *in vitro* and by senescent leaves *in vivo* indicates that dying cells can produce auxin; the sites of auxin production in the plant can be explained by the association of regions of meristematic activity with the cell deaths which occur as nutritive tissues regress and as vascular tissues differentiate. In the following sections the sites of auxin production will be discussed with these hypotheses in mind.

The best reviews on the production and distribution of auxin in the plant are by Söding (1952, 1961) but these, like many of the publications in this field, are in German. This important and interesting literature seems to be little known in English-speaking countries.

Investigations on auxin in plants have depended either on the use of extraction techniques, 'free' auxin being taken as that obtainable by short periods of extraction in the cold, or by the trapping of auxin diffusing from plant parts in agar. The disadvantage of the diffusion method is that it depends not only on the amount of auxin in the tissue but also on the auxin transport system; the disadvantage of extraction is that it may give a distorted picture of the amount of auxin available as a hormone, since some may be immobilized or compartmentalized within the tissue.

1. *Coleoptile tips*

Auxin was clearly identified as a plant hormone in the classical investigations of Went (1928) on the growth and tropisms of the coleoptiles of grass seedlings. Since that time the coleoptile has continued to occupy a central position in auxin research.

The classical work on coleoptiles, summarized by Went & Thimann (1937), established that the coleoptile tip is rich in auxin; that the growth of the coleoptile depends on a supply of auxin from the tip; that the auxin is transported basipetally from the tip in the polar auxin-transport system; and that tropic movements of the coleoptile could be explained by the asymmetric supply of auxin from the tip.

The decapitation of coleoptiles results in a cessation of growth; but after a few hours growth is resumed as a result of the 'regeneration of the physiological tip', a phenomenon whereby the apical region of the coleoptile stump becomes a source of auxin (Went & Thimann, 1937). This shows that the role of the coleoptile tip as an auxin source does not depend on any special anatomical features of the tip itself, but rather on its position at the apex of the coleoptile. The removal of the seed results in a decline in the amount of auxin in the coleoptile tip and also prevents the regeneration of the physiological tip (Skoog, 1937). The removal of the roots also results in a decline in the amount of auxin in the tip; the removal of both the seed and the roots results in a further decline (van Overbeek, 1937). These results indicate that the auxin economy of coleoptile tips depends on a supply of a substance or substances from the seed and that the roots are involved in this process in some way.

Germinating cereal seeds are rich in auxin and in bound forms of auxin, mostly IAA esters (Section II, 5). Pohl (1935, 1936) showed that a depletion of the amount of auxin in the seed resulted in a decline in coleoptile growth. He suggested that auxin from the seed moved acropetally in the vascular tissues and accumulated at the coleoptile tip. This conclusion was rejected by Skoog (1937) who was unable to detect auxin in agar blocks placed on the apical cut surfaces of coleoptiles. He postulated that a precursor of auxin moved from the seed to the coleoptile tip where it was converted into auxin. Van Overbeek (1941) and Wildman & Bonner (1948) found that excised coleoptile tips yielded several times more auxin by exhaustive diffusion into agar than could be obtained from tips by extraction immediately after excision. Their conclusions that coleoptile tips actually produce auxin depended on the questionable assumptions that diffusion and extraction were equally efficient and that no bacterial auxin production occurred during the prolonged diffusion periods under non-sterile conditions. However, even if appropriate corrections are made to their results, they still indicate that up to four times more auxin can be obtained by diffusion than by extraction (Sheldrake, 1973).

This production of auxin in coleoptile tips was interpreted as being due to the conversion of an auxin precursor supplied by the seed. Skoog (1937) showed that tryptamine could produce curvatures in coleoptiles similar to those induced by auxin but after a delay of several hours and concluded that it could act as an auxin precursor. There is, however, no evidence that it does so *in vivo*. Raadts & Söding (1957) found that both IAA and a labile substance which could undergo spontaneous conversion to IAA could be detected in coleoptiles. Chromatographic investigations of diffusates from coleoptile tips by Ramshorn (1955), Bohling (1959) and Shen-Miller & Gordon (1966) also showed that, in addition to IAA, at least one other compound with auxin activity in the coleoptile extension bioassay was present. This compound, called P by Shen-Miller & Gordon, is not transported in the polar auxin transport

system and is therefore inactive in the coleoptile curvature bioassay. Shen-Miller & Gordon (1966) showed that P was apparently converted to IAA by mild heat treatment and also that P and another compound with auxin activity in the coleoptile extension bioassay were converted to IAA in coleoptile tips. In some experiments the amounts of IAA increased considerably while the total amount of auxin activity in the coleoptile tips determined by the extension bioassay remained constant or even declined. By this criterion no auxin production could be said to have occurred; but the curvature bioassay, which detects only IAA, would indicate that auxin had been produced. Van Overbeek (1941) and Wildman & Bonner (1948) used the curvature bioassay; the auxin production they observed can therefore be equated with the production of IAA.

The substance in coleoptile tip diffusates identified chromatographically as IAA has recently been conclusively identified as IAA by mass spectrometry (Greenwood *et al.*, 1972).

The chromatography of ether extracts of germinating maize seeds (Hemberg, 1958) reveals the presence of IAA and other zones of auxin activity similar to those found in ether extracts or diffusates from coleoptile tips (Bohling, 1959; Shen-Miller & Gordon, 1966). If P and these other compounds represent precursors of IAA in the coleoptile tip and are also present in the seed, their movement from the seed to the coleoptile tip could explain the classical results on the auxin economy of coleoptile tips.

Guttation fluid from coleoptiles contains both IAA and other forms of auxin, including P and esters of IAA (Sheldrake, 1973). A similar pattern of auxin activity is found in the guttation fluid from primary leaves and from decapitated coleoptiles, showing that auxin is present in the xylem sap and is not merely eluted from coleoptile tips as guttation takes place. These results indicate that IAA and auxins inactive in the curvature bioassay (but active in the extension bioassay) move acropetally in the xylem from the seed. It can be shown by the use of dyes or radioactive IAA that substances moving in the xylem accumulate at the tips of coleoptiles, or indeed at the tips of the veins in any organ (Sheldrake, 1973). In decapitated coleoptiles substances accumulate at the apical part of the stump, although they are not detectable in agar blocks placed on the apical cut surface. This finding refutes Skoog's (1937) evidence against the acropetal movement of auxin.

The auxin economy of coleoptile tips can be explained as follows (Sheldrake, 1973): both free IAA and 'inactive' auxins move acropetally in the xylem from the seed to the coleoptile tip, or to the physiological tip of decapitated coleoptiles, where they accumulate. This process is affected by transpiration and root pressure, which may account for the influence of the roots on the amount of auxin in coleoptile tips. Both the free IAA and IAA released from 'inactive' auxins in the coleoptile tip can then be transported basipetally in the living cells of the coleoptile where it controls extension growth. The accumulation of auxin at the apical limits of the xylem could result in an asymmetry of auxin distribution since the anatomical distribution of the xylem in the extreme tip of the coleoptile is asymmetrical (Thimann & O'Brien, 1965). Such an asymmetric accumulation of auxin would account for the autonomous curvature

of coleoptiles which is observed when *Avena* seedlings are grown on a horizontal clinostat (Pisek, 1926; Lange, 1927).

The major unresolved problem is the identity of the 'inactive' auxins. Obvious candidates would be the IAA-inositol esters found in such large quantities in the seed. Perhaps compounds such as P detected after chromatography in ammoniacal solvents represent degradation products of IAA esters, formed either during the extraction procedures or as a result of ammonolysis.

The production of auxin from 'inactive' auxin in the coleoptile tip was referred to in the earlier literature as auxin *activation*, a term which emphasized the difference of this process from the *de novo* synthesis of auxin which takes place in other parts of plants. However in more recent, progressively simplified, accounts of this classical work, the production of auxin in coleoptile tips is described as auxin *synthesis* (e.g. Bonner & Galston, 1952; Leopold, 1964). The vicarious nature of the auxin economy of coleoptile tips is thus obscured and this has resulted in considerable confusion. For example, the fallacious conclusions of Winter (1966) referred to on p. 513 depended on the assumption that the coleoptile was a typical site of auxin biosynthesis. Problems of this sort disappear when it is remembered that there is no evidence for the *de novo* synthesis of IAA by coleoptiles *in vivo*.

2. *Young leaves, shoot tips and buds*

Avery (1935) was among the first to recognize the general pattern of auxin production by developing dicot leaves; in his studies on *Nicotiana* he found that "auxin is present only in growing leaves and that its concentration is roughly inversely proportional to the age of the leaf". Similar results have been obtained with leaves of *Phaseolus* (Shoji, Addicott & Swets, 1951; Humphries & Wheeler, 1964; Wheeler, 1968), *Solidago* (Goodwin, 1937), *Aster* (Delisle, 1937), *Coleus* (Jacobs & Morrow, 1957) and fronds of *Osmunda* (Steeves & Briggs, 1960). Auxin is produced throughout the period of leaf development in the basal meristematic region of the leaf of the unifoliate dicot, *Streptocarpus wendlandii*, while very little is present in the mature, apical parts of the leaf (Hess, 1958). Developing monocot leaves also contain more auxin in the basal meristematic region than in the rest of the leaf (van Overbeek, 1938).

Leaf development involves both meristematic activity and vascular differentiation. Jacobs & Morrow (1957) found a close correlation between auxin production and xylem differentiation in *Coleus* leaves and interpreted this as showing that the differentiation of xylem is controlled by auxin; but the results could also indicate that auxin is produced as a consequence of xylem differentiation: these interpretations are not mutually exclusive. Developing *Nicotiana* leaves contain more auxin in the veins than in the lamina (Avery, 1935) which might indicate that it is produced in the differentiating vascular tissue.

It is well known that relatively large amounts of auxin are produced by shoot tips (Thimann & Skoog, 1934; du Buy & Neurnbergk, 1935; Delisle, 1937; Söding, 1938; Eliasson, 1969) and developing buds (Czaja, 1934; Zimmerman, 1936; Söding, 1937; Avery, Burkholder & Creighton, 1937; Gunckel & Thimann, 1949; Dörffling, 1963).

Table 1. *The production of auxin by senescent leaves*

Species	Age of attached leaves (days)	Free auxin content, μg IAA equivalents/kg fresh weight	Author
Phaseolus vulgaris	19	9	Wheeler (1968)
	33	63	
	40	196	
	40 (shrivelled leaves)	332	
	Days after detachment of leaves		
Cucurbita pepo	0	0·5	Conrad (1965)
	11	50	
Phaseolus vulgaris	0	2	Sheldrake & Northcote (1968*c*)
	6	73	
Avena sativa	0	2	
	4	91	

The tissues used in all these investigations included not only the meristem but also the submeristematic region and young leaves and therefore contained both meristematic cells and differentiating vascular tissue.

3. *Senescent leaves*

The production of auxin by young, developing leaves declines as the leaves mature (see above). Shoji *et al.* (1951), on the basis of a single measurement of the auxin level in senescent leaves of *Phaseolus vulgaris*, concluded that a further decline occurred during leaf senescence. This conclusion appears to have been widely accepted, especially by workers on leaf abscission (e.g. Addicott, 1970). But there is now considerable evidence that auxin is produced as leaves senesce. This effect has been observed in *Bryophyllum crenatum* (Raadts, 1962), *Cannabis* (Conrad, 1962), *Cucurbita pepo* (Conrad, 1965), *Acer pseudoplatanus* (Dörffling, 1963), *Phaseolus vulgaris* (Sheldrake & Northcote, 1968*c*; Wheeler, 1968), *Avena sativa* (Sheldrake & Northcote, 1968*c*), *Hevea brasiliensis* (Chua, 1970) and *Prunus cerasus* (Kaska, 1972). The increases in auxin levels are large, often from 30- to a 100-fold (Table 1). The treatment of detached leaves with kinetin, which retards their senescence, results in a suppression of auxin production (Conrad, 1965). The production of auxin during senescence could account for the findings that *Bryophyllum* plants contain high levels of auxin in the autumn (Raadts, 1962) and that auxin levels increase considerably in plants kept in darkness for protracted periods (von Guttenberg & Zetsche, 1956).

The hydrolysis of proteins which occurs in senescent leaves results in elevated levels of free amino acids (Chibnall, 1939) including tryptophan (Commoner & Nehari, 1953; Pearse & Novelli, 1953; Lähdesmäki, 1968; Kim & Rohringer, 1969). The degradation of tryptophan and the consequent production of IAA could occur by transamination and possibly, in some species, to some extent by decarboxylation; the phenol/phenolase system could also be of some importance in cells in an advanced

state of disintegration. But the relative contributions of these pathways have not been investigated.

Measurements of the auxin in senescent leaves of *Acer pseudoplatanus* and several other species in the autumn revealed that the highest levels were present in leaves that were actually falling from the trees (A. R. Sheldrake, unpublished results). This auxin is therefore of no importance for the rest of the plant. But it seems possible that some of the auxin produced in senescent leaves could play a part in the control of abscission. Numerous investigations have shown that the application of auxin to petioles retards abscission (Addicott, 1970). The auxin of senescent leaves could therefore have an abscission-retarding effect as long as the transport system by which auxin moved down the petiole to the abscission zone continued to function. This might be of considerable physiological significance: leaves do not generally abscind until senescence is well advanced and it is this delay in abscission which enables nitrogenous and other compounds to be translocated back into the stem. Auxin produced in the senescent leaf might help to retard abscission until the transport systems themselves senesced. Thus the relatively small amounts of auxin which can be collected by diffusion from the petioles of senescent leaves (Böttger, 1970) reflect a decline of the auxin transport system rather than a decline in the amount of auxin in the leaves.

4. *Dicotyledonous seedlings*

Auxin is produced in the cotyledons of seedlings of *Lepidium* (van Overbeek, 1932), *Raphanus* (van Overbeek, 1933), *Lupinus* (Navez, 1933) and *Phaseolus* (Wheeler, 1968). The production of auxin in cotyledons (or in the endosperm of seedlings with endospermous seeds) can be explained as a consequence of the breakdown of reserve materials. The auxin production of cotyledons could also be regarded as analogous to the production of auxin by senescent leaves. The shoot tips of seedlings are sites of auxin production (Söding, 1952), as are shoot tips in general.

The demonstration that the growth of grass coleoptiles is controlled by auxin moving basipetally from the tip led to numerous attempts to explain the growth and tropisms of dicotyledonous seedlings in an analogous manner. In some species, e.g. *Raphanus* (van Overbeek, 1933) it was found that the removal of the cotyledons and the shoot tip inhibited the growth of the hypocotyl. But in other species such as *Lupinus* (Dijkman, 1934; Jahnel, 1937) and *Helianthus* (du Buy & Neurnbergk, 1932) decapitation had little or no effect on the growth of the hypocotyls. The attempt to explain all growth in terms of auxin meant that these results were interpreted by postulating that a diffuse production of auxin took place in the growing regions themselves (Jahnel, 1937; du Buy & Neurnbergk, 1935; Söding, 1952, 1961). However, Jost (1940) concluded from his studies on bean epicotyls that auxin alone could not be responsible for the control of growth and suggested that a second hormone was also involved. It is now known that gibberellins have striking effects on the growth of stems (Cleland, 1969). A widely used bioassay for gibberellins depends on the stimulation of hypocotyl growth by these hormones (Frankland & Wareing, 1960). The recent demonstration that gibberellins become asymmetrically distributed in shoot tips of *Helianthus* as a result of geotropic (Phillips, 1972*a*) and phototropic (Phillips, 1972*b*)

stimulation and the finding that stem growth in this species is controlled by gibberellin rather than auxin (Phillips, 1972*a*) mean that much of the early work on growth and tropisms in seedlings (on which the auxin theory of tropisms is based) must be reconsidered. In the light of these facts it no longer seems necessary to suppose that auxin is produced in the elongating regions of seedling stems.

5. *Stems*

Auxin is present in the basal meristematic region of growing monocot stems (Schmitz, 1933) where both meristematic activity and vascular differentiation are taking place. Mature stems of monocots (in which no secondary thickening occurs) contain little or no auxin (Schmitz, 1933). The growth of mature nodes can be resumed as a result of geotropic stimulation; in sugar cane, the intercalary zone grows from a few millimetres to more than a centimetre in a wedge-shaped way when the stem is placed horizontally. A marked increase in auxin occurs during this process (van Overbeek, Olivo & de Vasquez, 1945). It is interesting to note that these authors concluded that rather than being a cause, auxin was produced as a consequence of the geotropic response. Auxin production has also been observed in geotropically stimulated nodes in other grass species (Schmitz, 1933). Unfortunately in these reports no information was provided about the anatomical changes which accompanied the geotropic reaction and the associated production of auxin.

Auxin is produced in secondarily thickening dicot stems (Zimmerman, 1936; Söding, 1937, 1938, 1940; Jost, 1940; Allary, 1958; Hatcher, 1959; Dörffling, 1963; Sheldrake & Northcote, 1968*b*). The site of auxin production is the cambial region itself (Söding, 1937, 1938, 1940; Dörffling, 1963). In young leaves, developing buds, etc., a direct investigation of the cellular sites of auxin production has not been possible; but the cambial region has the advantage from an experimental point of view that the tissues can be separated easily, first by stripping off the bark and then by a selective scraping of tissues from the outside of the wood and the inside of the bark. In this way Söding (1937, 1938, 1940) showed that much more auxin was present in the cambium and its young derivatives than in the mature phloem and xylem tissues. Because he considered it intrinsically improbable that the differentiating vascular tissues could produce auxin, he concluded that the auxin was produced by the cells of the cambium itself. However, if the undifferentiated cambial cells are separated from the young, differentiating phloem and xylem tissues and their auxin contents are analysed separately, the highest amounts are found in the differentiating xylem cells, less in the cambium and least in the phloem (Sheldrake, 1971*a*). Unless there is a radial movement of auxin against a concentration gradient (which seems unlikely), these results suggest that auxin is produced in the differentiating xylem tissue rather than in the cambium.

The same conclusion has been reached by a different experimental approach. Segments of tobacco internodes maintained in sterile culture produce auxin and continue to do so for many months. This production of auxin is associated with continuing secondary thickening; if cambial activity is eliminated, auxin production ceases. In experiments with separated, regenerating bark tissue it was found that

auxin production depended on xylem differentiation, indicating that auxin is produced as a consequence of xylem differentiation (Sheldrake & Northcote, 1968*b*). The possibility that some auxin might also be produced in differentiating phloem tissue was not eliminated.

The continuing activity of the cambium in isolated stem segments in the absence of exogenous hormones (Jost, 1893, 1940; Sheldrake & Northcote, 1968*b*) indicates that the system is self-catalysing. Auxin is known to be involved in the control of cambial activity and vascular differentiation; it is also produced as a consequence of these processes. Some of the auxin produced in the cambial region is transported basipetally by the polar auxin transport system. In isolated stem segments this leads to considerably enhanced cambial activity towards the basal end of the explant and also to the formation of a basal callus (Jost, 1940; Sheldrake & Northcote, 1968*b*). By contrast, tobacco stem segments cultured in the presence of 2,3,5-tri-iodo benzoic acid, a specific inhibitor of polar auxin transport, exhibit pronounced cambial activity all along the explants, resulting in the production of serried ranks of tracheids which can exceed by several times the amount of xylem originally present (Sheldrake & Northcote, 1968*b*). This demonstrates very strikingly the 'positive feedback' inherent in the cambial system, which under normal circumstances must be 'damped' to some extent by the removal of auxin by polar transport.

6. *Roots*

Auxin is produced in isolated roots cultured *in vitro* (van Overbeek, 1939), but in the intact plant it is also transported from the shoot into the roots (McDavid, Sagar & Marshall, 1972) where it may be conjugated or destroyed. In *Lens* roots little auxin is found in the tip itself; most is found about 5 mm behind the tip (Pilet & Meylan, 1953) where xylem differentiation is taking place. Similarly, in *Zea* roots the tip contains less auxin than the region behind the zone of elongation, and nearly all the auxin is found in the stele rather than the cortex (Greenwood, Hillman, Shaw & Wilkins, 1973). The source of this auxin could be the differentiating vascular tissues, although some of it could have been transported acropetally in the stelar region. Other sites of cell death where auxin could be produced are the differentiating vascular tissues in the cambial region of secondarily thickening roots, the regressing root hairs and the root cap. The latter two may be of particular importance under non-sterile conditions.

Roots are very sensitive to exogenous auxin which is often present in soils in concentrations sufficient to affect them considerably (Section V). Therefore environmental as well as endogenous auxin could be important in the control of root growth under natural conditions.

7. *Flowers, fruits and seeds*

Auxin is produced in considerable quantities in developing flower buds; its production declines as the flower matures and little or none is formed by fully developed, unfertilized flowers (Söding, 1938; Kaldewey, 1959). The sites of auxin production in the earliest stages of flower development are not known, but it seems reasonable to

assume that expanding sepals and petals produce auxin in a manner analogous to young leaves. Auxin is produced in developing ovaries (Katunsky, 1936; Kaldewey, 1959) and here, as in the developing sepals and petals, both meristematic activity and vascular differentiation take place. Relatively large amounts of auxin are produced in anthers as the pollen develops (Weinland, 1941; Pilet, 1950; Hatcher, 1945) and as the tapetum regresses and dies. The auxin associated with the pollen of many species is probably formed during this process; the majority of the auxin of *Hibiscus* pollen and orchid pollinia is not inside the cells but in the fatty material between them (Laibach, 1932).

After pollination the pollen tubes grow down the style, sometimes causing a lysis of the cells between which they pass. Auxin is produced in the apical parts of *Nicotiana* styles soon after pollination and then later in the basal parts after the pollen tubes have penetrated them (Muir, 1942; Lund, 1956). Muir (1942) showed that no comparable auxin production occurred when pollen was germinated *in vitro* and concluded that auxin is produced in the style as a consequence of the release of hydrolytic enzymes by the pollen tubes. Auxin production begins in the ovary when the pollen tubes enter it (Muir, 1942; Lund, 1956) and fertilized, developing ovaries of many species have been shown to produce auxin (Söding, 1938; Dollfus, 1936; Gustavson, 1939; Borriss & Bussmann, 1939; Luckwill, 1948; Nitsch, 1952; Wright, 1956; Kaldewey, 1959). The development of the ovary involves the regression of a series of tissues, first the synergids and antipodal cells, then the nucellus and finally all or part of the endosperm. In a detailed anatomical study of rye grains, Nutman (1939) observed that "the initiation of each new phase of development occurs by the degeneration of some previously formed tissue" and suggested that these regressing tissues supplied not only nutrients but also hormones. Hatcher (1943) found that the production of auxin in rye grains was correlated with the regression of nutritive tissues and concluded that it was derived from the dying cells. Isolated embryos from developing cereal grains contain relatively little auxin; the great majority is found in the nutritive tissues (Hatcher & Gregory, 1941; Hatcher, 1943, 1945; Hemberg, 1955). In developing achenes of the strawberry (*Fragaria*) the levels of free tryptophan rise to a maximum as the nucellus regresses and large amounts of auxin are produced at this stage (Nitsch, 1952). Similarly, in developing apple seeds the degeneration of the nucellus is correlated with the production of high levels of auxin. Approximately eighteen times more auxin is present in the nutritive tissues than in the embryo (Luckwill, 1948).

Much of the auxin in developing cereal grains is converted to an esterified form from which IAA is released when the seeds germinate (Section II, 5) and bound auxins are also formed during the development of dicotyledonous seeds (Klämbt, 1960; von Raussendorf-Bargen, 1962; Zenk, 1964).

Auxin produced during the development of ovaries and seeds affects the movement of peduncles (Katunsky, 1936; Borriss & Bussmann, 1939; Kaldewey, 1959) and in some species has been shown to stimulate the development of fruit walls and receptacles (Nitsch, 1952). The growth of fruits is also influenced by gibberellins (Crane, 1964) which, like auxin, are produced in developing seeds (Cleland, 1969). The effects of the seeds on the development of the fleshy parts of the fruit are clearly

seen in apples with an asymmetric distribution of pips (Audus, 1953) and close correlations between the number of developing achenes and the growth of the receptacle have been demonstrated in the strawberry (Nitsch, 1950). But although hormones formed in the developing seeds influence the development of the fleshy parts of the fruit to a striking extent in some species, in others the developing fruit tissues may be more or less autonomous from a hormonal point of view. This must be the case in naturally parthenocarpic fruits such as seedless varieties of orange and cultivated bananas. In a number of other species whose fruits normally develop only after fertilization, parthenocarpy can be triggered off by wounding (Haberlandt, 1922), treatment with a variety of chemicals including synthetic auxins and gibberellic acid, or environmental influences such as exposure to cold (Nitsch, 1952). Once fruit development has been initiated it proceeds more or less normally, although parthenocarpic fruits are sometimes smaller than normal ones. The development of these fruits must depend on hormones produced within the fruit tissues themselves. In tomatoes, the production of auxin in parthenocarpic fruits has been directly demonstrated by extraction of the tissues of fruits induced to develop by phenylacetic acid (Gustavson, 1939) and by diffusion of auxin from fruits developing parthenocarpically after gibberellin treatment (Kurashai & Muir, 1962).

Fruit development takes many forms, but in fleshy fruits involves the expansion of cells formed before and sometimes after fertilization; in the larger fruits there is also considerable vascular differentiation. The highest amounts of auxin in parthenocarpically developing tomatoes are found in the tissues of the central axis and partitions (Gustavson, 1939) where vascular differentiation is pronounced. It seems possible that the differentiating vascular tissues could be a major site of auxin production in developing fruits, both parthenocarpic and normal.

8. *Cellular sites of auxin production*

It cannot be a general property of dividing cells to produce auxin, since most callus tissues cultured *in vitro* require auxin; if dividing cells produced it, these tissues would become autonomous with respect to auxin once cell division had been initiated. The hypothesis that auxin is produced by meristematic cells is not supported by the finding that meristematic embryos and cambial tissue contain less auxin than the dying tissues adjacent to them; and it is clearly unable to explain the production of auxin by senescent leaves. This hypothesis would require meristematic cells to contain elevated levels of tryptophan, for which there is no evidence. The only evidence in favour of the meristematic hypothesis of auxin production is the general correlation between regions of meristematic activity and auxin production, a correlation which can be explained equally well by the presence of dying cells. It is not possible to exclude the possibility that some meristematic cells produce auxin some of the time, but there is at present no reason to believe that this is the case.

The essential features of the hypothesis that auxin is produced as a consequence of cell death are that tryptophan is the limiting factor for auxin production; that in living cells the concentration of tryptophan is regulated and maintained at a level too low for the degradation of tryptophan, and hence the production of auxin, to occur to a

significant extent; and that autolysis results in increased levels of tryptophan. Some of the tryptophan released by autolysing cells may be converted to auxin by adjacent living cells; it is for this reason that I have referred to auxin production as taking place as a consequence of cell death, rather than simply in the dying cells themselves, although these may well be the major site of tryptophan degradation. With the possible exception of geotropically stimulated grass nodes, all the known sites of auxin production in plants are sites of cell death.

The sites of cytolysis within plants which, according to this hypothesis, are likely to be sites of auxin production are: regressing nutritive tissues (the tapetum, nucellus, endosperm and cotyledons), the dying cells of senescent leaves, possibly dying root hairs and root-cap cells, differentiating xylem cells, most differentiating fibres, possibly differentiating sieve tubes, and differentiating cork cells. Auxin is probably also produced by damaged and wounded cells (Section VIII).

IV. AUXIN PRODUCTION UNDER PATHOLOGICAL CONDITIONS

Attacks by fungi, bacteria and animals involve the death and breakdown of cells in the infected region; tryptophan released by the dying cells could be converted to IAA by enzymes of the host cells themselves or of the pathogen.

1. *Fungal and bacterial infections*

Tissues infected by bacteria or fungi usually contain considerably elevated levels of auxin (e.g. Wolf, 1956; Gruen, 1959; Sequeira, 1965; Kim & Rohringer, 1969) and tryptophan (Kim & Rohringer, 1969). Many fungal and bacterial pathogens are known to be able to convert exogenous tryptophan to auxin and it seems likely that they may play an important part in the production of auxin in infected tissues. By the use of rather dubious biochemical criteria, Sequeira (1965) attempted to determine the contributions of the host and the pathogen to auxin production in tobacco tissue infected by *Pseudomonas*; he concluded that both were important, the host being more so in the early stages of infection. But whatever are the relative contributions of host and pathogen, the production of auxin can be seen as a consequence of the release of tryptophan by the lysis and digestion of the infected cells.

2. *Animals*

Some animals which infect plant tissues feed by secreting digestive enzymes and then sucking up the digested material (Krusberg, 1963; Miles, 1968). As in fungal and bacterial diseases, it is possible that tryptophan released by proteolysis could be converted to auxin in the host as well as in the pathogen. Other animals digest the material internally and hence auxin is likely to be formed within the animal and may be released from it by excretion. For example, leaf-mining insect larvae which munch their way through the mesophyll deposit faecal pellets around which intumescences develop, probably as a response to auxin (La Rue, 1937). The application of mouse faecal pellets to mesophyll tissues has similar effects (La Rue, 1937).

These observations provide an approach to the understanding of galls produced by

more or less stationary animals which live entirely enclosed within the plant tissues. Animals whose posterior parts remain outside the plant do not produce galls (Mani, 1968). There is evidence that galls induced by *Meliodogyne* nematodes contain elevated levels of amino acids and auxin (Dropkin, 1969); it seems probable that auxin may also be produced in other galls. However, the wide diversity of galls suggests that hormones other than auxin are also involved; the balance and sequence of hormones released must be characteristic of the gall-inducing species.

3. *Viruses*

Unlike fungal, bacterial and animal pathogens, viruses do not sustain themselves by digesting the contents of the host cells but rather by perverting the metabolism of the cells to their own ends. Thus amino acids within the cells are used directly in the synthesis of viral proteins. Tryptophan is found in the protein of many plant viruses (Fraenkel-Conrat, 1968); its utilization in infected cells might be expected to lower rather than raise the levels of free tryptophan. This is indeed known to be the case in virus-infected potato tubers (Andreae & Thompson, 1950). It is therefore interesting to note that tissues infected with viruses generally contain less auxin than normal, uninfected tissues (Söding, 1961; Sequeira, 1963).

4. *Crown gall*

In many species, tumours develop in the vicinity of wounds infected by virulent strains of the crown gall bacterium, *Agrobacterium tumefaciens*. The proliferation of the tumour cells continues even if the bacteria are killed, showing that a permanent transformation, analogous to animal cancer, has taken place. The actual transforming agent may be a virus, carried as a temperate bacteriophage by *A. tumefaciens* (Beardsley, 1972). Sterile tumour tissue continues to proliferate when grafted into healthy plants and can also be cultured *in vitro* on a simple medium containing sugar and salts. These facts indicate either that crown gall tissues do not require auxins and other growth factors that are necessary for the growth of normal callus tissues, or that they have acquired the ability to produce them. The evidence is in favour of the latter explanation (for reviews see Beardsley, 1972; Wood, 1972). Non-sterile (Link & Eggers, 1941; Dye, Clark & Wain, 1961) and sterile (Kulescha, 1952) crown gall tissues have been shown to produce auxin. But they do not contain unusually high levels of amino acids in general (Lee, 1952) or tryptophan in particular (Henderson & Bonner, 1952).

All existing theories of crown gall seem to assume that the tissues contain a more or less homogeneous population of tumour cells (e.g. Braun, 1962; Wood, 1972). Braun (1958) has proposed that the systems synthesizing growth hormones are 'activated' in the transformed cells. An 'activation' of the auxin-synthesizing system would presumably involve an increased ability of the cells to convert tryptophan to auxin and/or an increased synthesis of tryptophan. But in fact crown gall tissues neither show an enhanced ability to convert tryptophan to auxin (Kulescha, 1952) nor do they contain more tryptophan than normal callus tissues (Henderson & Bonner, 1952). It is therefore necessary to question Braun's assumptions.

Perhaps crown gall tissues contain a more or less stable mixture of cells, some trans-

formed, others normal. Even 'clones' grown from single cells can contain a mixture of normal and transformed cells, since reversion is known to be possible (Braun, 1959). The differences between partially and fully transformed tissues (Braun, 1962) could be explained on this basis in terms of the former having a lower proportion of transformed cells. Furthermore, only some of the transformed cells may be involved in the production of the hormones to which the other cells respond. This would be possible, for example, if at any given time some of the transformed cells died, with the consequent production of auxin and also, possibly, cytokinins (Section VII, 3). Crown gall tumours do in fact contain considerable numbers of dead and dying cells; it has often been observed that cell divisions occur in crown galls around necrotic areas or zones of tracheid differentiation (Robinson & Walkden, 1923; Banfield, 1935; Manignault, 1953; Therman, 1956; Kupila, 1958). No comparable histological studies of crown gall tissues cultured *in vitro* have been published, but in all the cultures which I have examined dead cells are quite common. The tumourous transformation viewed in this light would not involve an 'activation' of the auxin synthesizing system in all the cells but would lead to auxin production by causing a more or less constant percentage of the cells to die. This could be thought of by analogy with lysogenic bacterial cultures where at any given time a minority of the cells are killed by bacteriophages which in the other cells are integrated with the genome and remain latent.

The autonomy of 'habituated' tissues which arise spontaneously from normal calluses after more or less prolonged periods in culture might be explicable in a similar way. But in the absence of any quantitative data on cell death within these tissues, or indeed within normal callus tissues, the hypotheses advanced above can be no more than speculative.

V. ENVIRONMENTAL AUXIN

Auxin has been detected in a wide variety of soils (Parker-Rhodes, 1940; Stewart & Anderson, 1942; Hamence, 1944, 1946; Whitehead, 1963; Sheldrake, 1971*b*). It is produced from tryptophan by many soil micro-organisms (Roberts & Roberts, 1939) and is found in the highest amounts in soils rich in decaying organic matter. The auxin content of the soil represents an equilibrium between production and destruction (Parker-Rhodes, 1940; Hamence, 1946) and is presumably also affected by factors such as rain and leaching.

The elongation of root hairs can be stimulated by extraordinarily low concentrations of auxin: 1×10^{-4} μg/l is sufficient to bring about a significant effect (Jackson, 1960). The growth of roots themselves is also sensitive to exogenous auxin, often being inhibited by concentrations in excess of about 10 μg/l (Whitehead, 1963). Thus roots grown in this order of auxin concentration have a more dense covering of root hairs which are also longer (Ekdahl, 1957). The concentration of auxin in the soil solution usually lies in the range of 1–50 μg/l (Whitehead, 1963; Sheldrake, 1971*b*). These are average values; it should be remembered that the soil is made up of many micro-environments where locally higher or lower concentrations may be present.

The effects of auxin on roots may well be of adaptive significance; the relatively

high concentrations of auxin associated with decaying organic matter could cause the absorptive area of the growing roots to be increased where nutrients are most readily available. For similar reasons, environmental auxin may also be important for bryophytes which grow in close association with their substratum. Auxin above about 10 μg/l induces rhizoids in liverworts; a variety of substrata supporting the growth of bryophytes have been found to contain concentrations of auxin in this range (Sheldrake, 1971*b*).

VI. AUXIN AND LOWER PLANTS

Ideas derived from the study of the hormonal role of auxin in vascular plants have been extrapolated to algae, bryophytes and fern gametophytes on the assumption that auxin is synthesized in meristematic cells. But attempts to explain apical dominance and the control of growth in these plants in terms of auxin may be based on a false analogy if auxin is produced in higher plants as a consequence of cytolysis. Demonstrations that non-vascular plants are able to form auxin from exogenous tryptophan (e.g. Libbert *et al.*, 1966; Ahmad & Winter, 1969) do not prove that they normally produce auxin or that auxin is a hormone in these plants.

Small amounts of auxin have been detected in a variety of algae (Conrad & Saltman, 1962), but the use of non-sterile material renders these results equivocal since auxin can be produced by micro-organisms epiphytic on algae (Libbert *et al.*, 1966). Several reports that IAA supplied in ethanolic solutions enhances the growth of algae have been shown to be due to the ethanol rather than the IAA (Bach & Fellig, 1958; Street *et al.*, 1958). Other investigations of the effects of IAA have involved the use of unbuffered solutions where the relatively high amounts of IAA used may have lowered the pH and thus affected the algae in a rather unspecific way (e.g. Jacobs, 1951). In short, there seems to be no convincing evidence that auxin has specific effects on algae and still less that it is an endogenous hormone.

The evidence for auxin as an endogenous hormone in bryophytes and fern gametophytes is equally unconvincing. There is no persuasive evidence that it is produced in sterile tissues of these plants and attempts to demonstrate that apical dominance and other inhibitory phenomena are controlled by auxin have involved the use of high and probably toxic concentrations of IAA (for references see Sheldrake, 1971*b*). The only well-established positive response to auxin which occurs at low concentrations is the stimulation of rhizoid development in liverworts. This can be explained in terms of a reaction to exogenous, environmental auxin (Section V).

This response may have evolved in the liverworts as an adaptive reaction for the absorption of nutrients, with auxin acting as a messenger of decomposition and decay. It could perhaps provide a clue for the understanding of the evolutionary origin of auxin as a hormone in higher plants (Sheldrake, 1971*b*). If the precursors of the vascular plants had evolved a similar response, auxin would have been in a suitable position to become an endogenous hormone when cell death and auxin production within the plant became an integral part of development with the evolution of the vascular system. Seen in this light, the response of roots and root hairs to environmental auxin might represent an evolutionarily primitive characteristic which has been adapted and retained.

VII. THE PRODUCTION OF OTHER PLANT HORMONES

This subject will not be discussed at such length as the production of auxin, partly because the literature is more recent, smaller and less tangled; partly because several recent reviews are available: on cytokinins by Fox (1969), Skoog & Armstrong (1970) and Kende (1971); gibberellins by Cleland (1969), Lang (1970) and West (1973); abscisic acid by Addicott & Lyon (1969) and Dörffling (1971); and on ethylene by Burg (1962), Pratt & Goeschl (1969), Mapson (1969) and Abeles (1972). I shall concentrate on those aspects of the literature that have a bearing on the understanding of the sites of production of these hormones: as with auxin, it is only when the cellular sites of synthesis are known that a clearer understanding of the control of hormone production will become possible.

In the light of the evidence in favour of auxin production as a consequence of cell death, it seems worth considering the possibility that other hormones might also be formed as a result of cytolytic processes. There is no *a priori* reason why they should be; but conversely there is no *a priori* reason for assuming that they are synthesized in living cells. In the following sections I have attempted to weigh up the evidence for and against these possibilities. On balance it seems probable that abscisic acid is synthesized by living cells; that the synthesis of gibberellin precursors occurs in living cells, but that the final oxidative reactions necessary for the production of these hormones might take place as a consequence of cell death; that cytokinins though produced by living cells, at least in root tips, are also formed by dying cells; and that ethylene is produced as a consequence of cell damage and cell death.

1. *Abscisic acid*

Two pathways of abscisic production have been proposed: by direct synthesis from mevalonate or by the oxidative breakdown of carotenoids (Addicott & Lyon, 1969). The photo-oxidation of violaxanthin results in the production of xanthoxin, a naturally occurring compound closely related to abscisic acid and with similar biological activity (Taylor & Burdon, 1970).

Abscisic acid has been found in a wide range of plants and in a variety of tissues (Milborrow, 1967, 1968). It is present and probably produced in the shoots of seedlings (Teitz & Dörffling, 1969), in young leaves and buds (Milborrow, 1967) and in developing fruit tissue (Rudniki, Pieniazek & Pieniazek, 1968; Rudniki & Pieniazek, 1970; Dörffling, 1971; Davis & Addicott, 1972). A striking increase in abscisic acid occurs in leaves subjected to water stress (Wright & Hiron, 1972) and mature leaves of trees produce abscisic acid in response to short-day conditions (Phillips & Wareing, 1959). Abscisic acid is produced in considerable quantities in senescent leaves (Chin & Beevers, 1970; Böttger, 1970) and in ripening and senescent fruit tissues (Rudniki, Machnik & Pieniazek, 1968; Goldschmidt, Eilati & Goren, 1972; Davis & Addicott, 1972).

Taylor & Smith (1967) suggested that abscisic acid is produced *in vivo* by the photo-oxidative breakdown of carotenoids, especially in senescent leaves. This hypothesis at first sight seems to provide a plausible explanation for the increased abscisic acid

production which occurs when etiolated tissues are exposed to light (Wright, 1968). However, light brings about many changes in etiolated tissues and there is no reason to suppose that the influence of light on abscisic acid production is direct. There is in fact no evidence for the production of abscisic acid from carotenoids *in vivo*; and the available evidence seems to be against it. In pea seedlings treated with gibberellic acid no relationship could be found between abscisic-acid production and changes in the amounts of carotenoid pigments (Tietz & Dörffling, 1969). There is evidence that abscisic acid is produced *de novo* in ripening strawberries, rather than by the breakdown of carotenoids (Rudniki & Antoszewski, 1968). The direct synthesis of abscisic acid from mevalonate has been demonstrated in wilting leaves (Milborrow, 1972) and in this case again the evidence is against carotenoids acting as precursors. Abscisic acid is produced by senescent leaves (Chin & Beevers, 1970) and ripening fruits (Rudniki, Machnik & Pieniazek, 1968) in the dark. Thus although the photo-oxidation of carotenoids may result in the formation of growth inhibitors under some circumstances, it does not appear to be the major pathway of abscisic-acid production in the plant, even in senescent tissues; the predominant route of abscisic-acid production *in vivo* may well be the direct one. This *de novo* synthesis of abscisic acid presumably occurs only in living cells.

2. *Gibberellins*

Gibberellins were first isolated from fungi; they are also produced by some bacteria (Vančura, 1961) and have been detected in algae (Radley, 1961). In fungi and in higher plants they are known to be formed from (−) kaurene which is in turn derived from mevalonate (Lang, 1970; West & Fall, 1972). A number of inhibitors (e.g. Amo 1618) which block the synthesis of kaurene prevent the production of gibberellins (Dennis, Upper & West, 1965) and when applied to higher plants act as growth retardants. The conversion of kaurene to gibberellic acids involves a series of oxidative reactions which are little understood (West & Fall, 1972; West, 1973).

Gibberellins are produced in young, developing leaves of *Phaseolus* (Humphries & Wheeler, 1964), *Helianthus* (Jones & Phillips, 1966) and *Taraxacum* (Fletcher, Oegema & Horton, 1969). The levels of gibberellins in mature leaves are low. In *Helianthus* shoot tips, gibberellin production occurs in the young leaves rather than in the meristem itself (Jones & Phillips, 1966). Gibberellins have been detected in the xylem sap of a number of species (e.g. Phillips & Jones, 1964; Skene, 1967) indicating that they are produced in roots; and the production of gibberellins by root tips + caps of *Helianthus* has been directly demonstrated (Jones & Phillips, 1966). There is indirect evidence that they are produced in the cambial region. The application of auxin to woody stems induces cambial development and tracheid differentiation; but for normal vessel differentiation another hormone is necessary. This second hormone was shown by Rehm (1936) and Jost (1940) to be formed not only in young leaves but also as a consequence of cambial activity. In the presence of auxin the effects of gibberellic acid on the differentiation of cambial derivatives (Wareing, Hanney & Digby, 1964) are the same as those attributed by Rehm and Jost to the unknown hormone, suggesting that it was in fact gibberellin. Gibberellins are

formed in considerable quantities during seed development (Cleland, 1969), by far the largest amounts being produced as the nucellus and/or endosperm degenerate (Corcoran & Phinney, 1962; Jackson & Coombe, 1966; Luckwill, Weaver & MacMillan, 1969; Chacko, Singh & Kachru, 1970). In developing seeds of *Echinocystis* the great majority of the gibberellin is found in the nutritive tissues rather than in the embryo (Corcoran & Phinney, 1962). As seed development proceeds towards dormancy, free gibberellins are converted to bound forms, as glycosides of gibberellic acids (Barendse *et al.*, 1968; Barendse, 1971; Sembdner *et al.*, 1972). In a number of dicotyledonous species, free gibberellins have been shown to be released from these bound forms after germination of the seeds; and at least the early stages of seedling development are independent of *de novo* gibberellin synthesis (Barendse *et al.*, 1968; Dale, 1969). Similarly, the 'production' of gibberellins by newly germinated barley embryos may be due to their release from a bound form (Cohen & Paleg, 1967). The release of free gibberellins from a bound form has also been shown to occur in etiolated wheat leaves soon after an exposure to red light (Loveys & Wareing, 1971).

Taken as a whole, these observations would seem to exclude the possibility that meristematic tissues are major sites of gibberellin production: the apical meristem of *Helianthus* does not produce gibberellins; the meristematic embryonic tissues of developing seeds contain relatively little gibberellin and the apparent production of gibberellins by germinating embryos can be explained in terms of a release from a bound form. Growing cells in general also seem unlikely to be sites of gibberellin synthesis, otherwise these hormones would not be a limiting factor for the growth of stems. The only feature which all the sites of gibberellin production have in common is the presence of dying cells; in root caps, in regressing nutritive tissues and in differentiating vascular tissue. In this connexion it is interesting to note that gibberellins are produced at or near the wounded surfaces of potato and Jerusalem artichoke tuber tissues shortly after cutting (Rappaport & Sachs, 1967; Kamisaka & Masuda, 1968; Bradshaw & Edelman, 1969). The simplest hypothesis suggested by these data is that gibberellins are produced as a consequence of cytolysis. This suggestion may seem both surprising and improbable from a biochemical point of view. But it is not necessary to suppose that gibberellins are synthesized from mevalonate in dying cells; more immediate precursors such as kaurene or kaurenoic acid could already be present and then only the final oxidative reactions necessary for the conversion of these compounds to gibberellins need take place as a consequence of cell death. This hypothesis appears to be in conflict with the finding that gibberellin levels decline rather than increase in senescent leaves (Fletcher *et al.*, 1969; Chin & Beevers, 1970) and fruits (Goldschmidt *et al.*, 1972) but perhaps in these cases the cells do not contain the necessary precursors.

Fungal cultures do not produce gibberellins in significant quantities until the phase of exponential growth has ceased. There is, however, some evidence that gibberellins are produced in the stationary phase metabolically rather than autolytically, since autolysis within these cultures does not become apparent until a rather later stage (Borrow *et al.*, 1955; Jefferys, 1970). It is difficult to assess the relevance of these findings to gibberellin production in higher plants, but they perhaps weaken the case

for thinking that gibberellins are produced as a consequence of cell death, or at least of autolytic processes.

3. *Cytokinins*

A diphenyl urea isolated from coconut milk has cell-division-stimulating activity (Shantz & Steward, 1955); and cell division factors, thought to be nicotinamide derivatives, were isolated from several types of cell cultured *in vitro* (Wood, Braun, Brandes & Kende, 1969). These compounds have now been re-identified as purinones (Wood, 1970). With these exceptions, the naturally occurring cytokinins are adenine derivatives, found in plants as free bases and as ribosides and ribotides. The same or closely related compounds have been found in the transfer RNA (tRNA) of animals, bacteria, fungi and higher plants (Skoog & Armstrong, 1970; Kende, 1971). There is strong evidence, summarized by Kende (1971), that cytokinins in tRNA are synthesized by the attachment of an isopentenyl group, derived from mevalonate, to adenine in preformed tRNA, that plant tissues requiring an exogenous supply of cytokinin as a growth factor are capable of synthesizing cytokinins in tRNA and that free cytokinins are not involved in any direct way in the formation of cytokinin nucleotides in tRNA. But while the cytokinins of tRNA are not derived from free cytokinins, the free cytokinins could be derived from the cytokinins in tRNA by hydrolysis (Sheldrake & Northcote, 1968*b*). Hydrolysates of tRNA from animals, micro-organisms and plants are active in cytokinin bioassays (Bellamy, 1966; Skoog *et al.*, 1966; Letham & Ralph, 1967).

Cytokinins are found and probably produced in developing fruits and seeds, particularly in the nutritive tissues (Steward & Shantz, 1959; Skoog & Armstrong, 1970), in germinating seeds (Barzilai & Mayer, 1964), in young leaves and buds (Engelbrecht, 1971), developing flower petals (Mayak, Halevy & Katz, 1972), root tips + caps (Weiss & Vaadia, 1965; Short & Torrey, 1972*a*) and in the cambial region (Bottomley *et al.*, 1963; Nitsch & Nitsch, 1965). Although the hydrolysis of tRNA with the release of cytokinin ribotides, ribosides and free bases might take place in living cells, it would be almost inevitable in dying, autolysing cells. The tissues in which cytokinins are produced contain dying cells, either in regressing nutritive tissues, in root caps or in differentiating vascular tissues; in the latter case RNA breakdown takes place not only as xylem cells and fibres differentiate and die, but also during sieve-tube differentiation which involves the loss of the nucleus, ribosomes and most cell organelles (Northcote & Wooding, 1968).

The production of cytokinins by autolysing cells might lead to an apparently paradoxical situation in senescent leaves since these hormones are known to retard leaf senescence in many species. Nevertheless, there is evidence that a cytokinin is produced in senescent leaves of *Populus* and *Acer* (Engelbrecht, 1971). In senescent leaves which contain low levels of cytokinins, any cytokinin production which occurs may be masked by a rapid destruction and metabolism of the hormones (Srivastava, 1968). There is evidence that cell-division-stimulating substances are produced in the yellowing regions of slowly senescing cherry laurel leaves; Godwin (1926) observed zones of cell division on the outskirts of the yellowing regions, resembling those that

are formed around wounded regions in the leaves of this species (Blackman & Matthaei, 1901). When yellowing began behind these zones, another wave of division sometimes occurred, again parallel to the advancing front of yellowing. These findings might indicate that cytokinins are released by the senescent cells. The production of cytokinins by wounded cells in senescent leaves is implied by the finding that the regions immediately adjacent to cuts in leaves of cherry laurel, *Narcissus* and several other species remain intensely green while the surrounding areas turn yellow (Godwin, 1926). The 'green islands' in senescent leaves around diseased areas point to the production of cytokinins in the infected regions (Mothes, 1970) and indeed in some cases high cytokinin levels in green islands have been directly demonstrated (Engelbrecht, 1968).

Cytokinins are produced by crown gall tissue (e.g. Tegley, Witham & Krasnuk, 1971) and certain types of callus tissue (Miura & Miller, 1968; Short & Torrey, 1972*b*; MacKenzie & Street, 1972; Dyson & Hall, 1972) cultured in the absence of cytokinins. In suspension cultures of pea (Short & Torrey, 1972*b*) and *Acer* (MacKenzie & Street, 1972) calluses, cytokinins are formed during the lag phase preceding cell division and reach a maximum at about the time that cell division is initiated. The cytokinin levels then decline during the period of most active growth. The initiation of cell division occurs as a response to cytokinins and only takes place if a critical level is reached (MacKenzie, Konar & Street, 1972). It seems possible that autolysing cells within these cultures could be a source of cytokinins, but unfortunately no data on the numbers of dying cells are available. Anyone who has examined tissue cultures under the microscope will know that dead cells are far from rare.

There is some important evidence that autolysis alone cannot account for cytokinin production by pea root tips+caps (Short & Torrey, 1972*a*) and cultures of the bacterium *Rhizobium* (Phillips & Torrey, 1972) since more free cytokinin is found than can be accounted for in tRNA. Pea root tips contained up to 27 times more free (ethanol extractable) cytokinin than in the tRNA and *Rhizobium* cultures released about 3 times more into the medium than was found in the soluble RNA of the cells. These comparisons did not involve any corrections for losses of RNA during extraction and purification. The RNA from pea root tips was more highly purified (involving several more purification steps) than that from *Rhizobium*. But unless the recoveries were very low these results suggest that cytokinins can be produced by living cells, implying either a rapid turnover of tRNA or an actual direct synthesis of free cytokinins and cytokinin ribosides. The terminal millimetre of root tips contains over forty times more cytokinin than the immediately subjacent parts of the root (Weiss & Vaadia, 1965; Short & Torrey, 1972*a*) suggesting that the cytokinins are produced either in the meristematic region or in the root cap, or in both. Root tips are probably a site of cytokinin production of special importance for the whole plant since cytokinins are carried from the roots to the shoots in the xylem sap (Kende, 1971). Only further quantitative investigations will reveal the extent to which living cells produce cytokinins in other parts of the plant and the relative contributions of living and autolysing cells to cytokinin production.

It seems very probable that cytokinins are released from the tRNA of autolysing

micro-organisms, decaying organic matter, etc., in the soil. Presumably they are also degraded in the soil; but perhaps the levels of environmental cytokinins might be sufficient to influence parts of plants sensitive to exogenous cytokinins. For example, the induction of buds on moss protonemata by low levels of cytokinins (Bopp, 1968) could represent an adaptive response to environmental cytokinins analogous to the adaptive response of liverworts to environmental auxin (Sheldrake, 1971*b*).

4. *Ethylene*

Several possible precursors of ethylene in the plant have been proposed (Mapson, 1969), but the most probable is methionine (Yang & Baur, 1972). Methionine at the C-terminal end of peptides produced by proteolysis can also act as an ethylene precursor (Demorest & Stahmann, 1971). Ethylene can be released from methionine non-enzymically by the action of hydrogen peroxide in the presence of certain metal ions, and, *in vitro*, by the action of peroxidase, but the enzymic mechanism of ethylene production *in vivo* is not yet known (Yang & Baur, 1972; Abeles, 1972).

A burst of ethylene production occurs soon after plant tissues are wounded (Burg, 1962) and considerable quantities are produced in diseased tissues (Williamson, 1950). Under normal conditions ethylene is produced by senescing leaves (Morgan, Ketring, Beyer & Lipe, 1972), senescing petioles (Rubinstein & Abeles, 1965), senescing flower petals (Mayak, Halevy & Katz, 1972), ripening fruits (Burg, 1962) and germinating seeds (Spencer & Olson, 1965). It is also produced in young, growing tissues (Burg, 1968) in which vascular differentiation is taking place. The application of toxic compounds such as copper sulphate to plant tissues leads to 'stress-induced' ethylene production (Abeles & Abeles, 1972). Ethylene production is also induced by the administration of unphysiologically high concentrations of auxin (e.g. Chadwick & Burg, 1967; Burg & Burg, 1966) probably as a result of non-specific damage (Muir & Richter, 1972). A rise in ethylene production follows the irradiation of fruits or vegetative tissues (Pratt & Goeschl, 1969). In all these cases ethylene production could be explained as a consequence of cell damage, senescence or death.

Ethylene production is increased when *Coleus* (Abeles & Gahagan, 1968) and tomato (Leather, Forrence & Abeles, 1972) plants are placed horizontally. In the latter case the plants were rotated on a clinostat; the increased ethylene production was therefore unlikely to be a consequence of the geotropic response. It seems possible that these observations could be explained as instances of stress-induced ethylene production as a result of literal, physical stress.

Ethylene is produced in the soil by micro-organisms (Lynch, 1972) and some soils contain sufficient quantities to affect the growth of roots (Smith & Russell, 1969).

VIII. THE WOUND RESPONSE

It has long been known that cells adjacent to wounded or necrotic areas react to form a protective layer. The nature of the wound response depends on the tissue and on factors such as humidity and osmotic pressure (Lange & Rosenstock, 1963). In many cases it involves cell division with the plane of division roughly parallel to the wound (Bloch, 1941, 1952). Haberlandt's (e.g. 1913, 1914, 1921, 1922) classical

studies showed that the wound response was influenced by substances released by the damaged cells which he called wound hormones, or more generally necrohormones. The more pronounced wound response in the neighbourhood of vascular bundles was interpreted by Haberlandt (1914) to be due to 'leptohormones' diffusing from the vascular tissues. Cell division around dead or dying cells has been observed in many other situations, for example in tissue cultures (Jones, Hildebrand, Riker & Wu, 1960), crown gall tumours (Section IV, 4), 'genetic' tumours of *Nicotiana* (Hagen, Gunckel & Sparrow, 1961) and around necrotic areas infected with viruses (Esau, 1938).

Cell division around wounded areas usually ceases after a few days; the wound response is self-limiting. This shows that the dividing cells do not themselves produce the necessary stimulus for cell division, but that cell division depends on the wound stimulus. It is interesting to imagine what would happen if the cells around the wounded area possessed some heritable instability (e.g. as a result of a 'lysogenic' type of virus infection) such that some of them died after dividing. In this case a new wound response would take place around the dying cells and then, after this new wave of division, further cell death might ensue, and hence further division, and so on. The result would be an autonomous, tumourous tissue. This is essentially the mechanism proposed for crown gall in Section IV, 4.

Haberlandt (1928) suggested that necrohormones might be of importance in the initiation of the periderm, which often occurs below necrotic areas where hair cells have died or where epidermal cells are disrupted by the growth in circumference of the stem. He was unable to explain the continued activity of the cork cambium once it had been initiated. However, by a simple extension of the necrohormone concept this could be seen as a consequence of the differentiation of the cork cells, which die as they differentiate. Similarly, the division of the cells of the vascular cambium adjacent to differentiating xylem cells provides a striking analogy to the wound response. Thus the idea that hormone production occurs as a consequence of cell death can be arrived at independently of a knowledge of the chemical identity of the hormones.

A substance capable of inducing cell division in bean pods was isolated by Bonner & English (1938) and given the name traumatin. But the bean-pod assay is very unspecific and traumatin is inactive in other cell-division assays (Fox, 1969); it is probably of little importance in the wound response in most tissues and even in bean pods its physiological significance is far from clear.

Haberlandt never identified the necrohormones but it now seems likely that wounded cells could be a source of auxins, cytokinins, gibberellins and ethylene. Auxin is known to be produced as a consequence of wounding (Hemberg, 1943); so are gibberellins (Section VII, 2), ethylene (Section VII, 4) and, probably, cytokinins (Section VII, 3). The production of some or all of these hormones by wounded cells could explain the effects of necrohormones; for example, a combination of auxin and cytokinins is known to stimulate cell division in a variety of tissues (Fox, 1969). The production of wound hormones and the normal production of hormones as a consequence of cell death can be seen as two aspects of the same phenomenon (Sheldrake & Northcote, 1968*a*).

IX. THE CONTROL OF HORMONE PRODUCTION AND DISTRIBUTION

In living cells the control of biosyntheses depends on the availability and compartmentalization of substrates and co-factors, on the control of enzyme synthesis at the transcriptional and translational levels, and on feedback mechanisms involving allosteric enzymes. Factors such as these presumably control the production of hormones (e.g. abscisic acid) that are made in living cells. But beyond this vague and unspecific statement, at present no more can be said.

On the other hand, it is possible to understand, at least in general terms, the control of hormone production that occurs as a consequence of cell death; this depends on the control of cell death itself. The notion that compounds as important as hormones are normally produced by dying cells may at first sight seem improbable on the grounds that insufficient control would be possible. But this difficulty is illusory: the differentiation of vascular tissues, the regression of nutritive tissues and the senescence of leaves and other organs do not take place at random. And the biochemical changes in dying cells occur in a definite and controlled sequence.

The idea that cell differentiation and cell death are controlled by hormones which are themselves produced in dying, differentiating cells may appear paradoxical. But in fact it is not at all surprising. Plant development is an autocatalytic process; the control of growth and differentiation depends on the production of plant hormones; hormone production must in turn be a consequence of growth and differentiation.

The cells of nutritive tissues, of senescent leaves, differentiating xylem cells, fibres and cork cells undergo a progressive autolysis and disintegration as they die. To start with they are living; at the end of these processes they are dead; in between they are dying. At exactly what stage they could first be said to be dying is a semantic question which it does not seem very fruitful to pursue. However, this point is more interesting when considering sieve tube differentiation, which involves a controlled, partial autolysis. Many of the biochemical changes that occur in the early stages of cell death in other cells may take place during the differentiation of sieve tubes, although the resulting cells are semi-living. But even in parenchymatous cells a turn-over of cell constituents takes place. Whole organelles such as mitochondria are broken down in vacuoles, which can be regarded as lysosomes (Mathile, 1969). Thus some of the autolytic processes that occur on a large scale when a whole cell dies may be taking place on a smaller scale within living cells. And a sublethal cytolysis may also occur in cells which are damaged, but not badly enough to kill the cell. Haberlandt (1922) observed cell divisions in damaged cells which he attributed to wound-hormone production without cell death.

Many of the arguments advanced above in favour of hormone production by dying cells would also apply to living cells in which a sublethal autolysis was taking place; this might be of particular importance in the understanding of cytokinin and stress-induced ethylene production. Hormone production as a consequence of cell death could be seen as an extreme case of hormone production by autolytic processes which may occur to some extent in living cells.

The distribution of plant hormones depends not only on the amounts produced

and on the sites of production, but also on their movement and destruction. Auxin moves in a specific transport system basipetally in shoots and acropetally in roots (Goldsmith, 1969). There is evidence that gibberellins (Jacobs, 1972) and abscisic acid (Milborrow, 1968) can move basipetally in shoots. Both auxin and gibberellins (Phillips, 1972*a*, *b*) become redistributed in tissues as a result of phototropic and geotropic stimulation. Gibberellins (Phillips & Jones, 1964; Skene, 1967) and cytokinins (Kende, 1971) move from the roots to the shoots in the xylem. Little or no auxin is found in the xylem sap of trees, which contains auxin-destroying enzymes (Sheldrake & Northcote, 1968*d*). Gibberellins (Kluge, Reinhard & Ziegler, 1964) and abscisic acid (Bowen & Hoad, 1968) have been detected in phloem sap. These movements account for the effects of hormones in regions remote from their sites of production; but it is probable that their movement over short distances by diffusion is important for the control of processes which occur in the immediate environment of hormone-producing cells; for example, cell division and new xylem differentiation in the neighbourhood of differentiating xylem cells (Sheldrake & Northcote, 1968*b*).

Little is known about hormone destruction and immobilization *in vivo*, although it is clear that under some circumstances the conjugation of auxin, abscisic acid and gibberellins is important in the regulation of hormonal levels. A great deal of work on the destruction of auxin by oxidases in plant homogenates has been carried out (e.g. Galston & Hillman, 1961) but its relevance to the destruction of auxin *in vivo* is obscure. Indeed the extent to which endogenous hormones are actually destroyed rather than conjugated, lost from the plant (e.g. hormones in abscinding leaves; ethylene escaping by diffusion) or simply diluted by growth is not known.

X. CONCLUSION

All sorts of biochemical processes take place when cells die and autolyse. Proteins are hydrolysed, releasing peptides and amino acids; amino acids are degraded to a variety of different products; nucleic acids are hydrolysed to oligonucleotides, nucleotides, nucleosides and free bases; lipid membranes disintegrate, releasing enzymes, substrates and salts that were previously localized within cellular compartments; oxidation reactions occur (for example in the browning of cut potato and apple tissues) which would not take place in living cells. Autolysing cells must release a great variety of compounds into their immediate environment and it seems almost inevitable that nearby living cells would be influenced in some way. Cells die as they differentiate in all vascular plants; cells die in all organisms as a result of wounding and infection; cell death occurs in animals in some types of differentiation (e.g. of keratinized skin cells) and as cells are turned over in most mature tissues; many cancerous tumours in animals contain considerable numbers of dead and dying cells; and several tissues or groups of cells regress and die during the development of animal embryos. It is perhaps significant that a number of substances with hormonal activity in animals are, like IAA, breakdown products of amino acids: tyramine, tryptamine, 5-hydroxytryptamine and histamine. The latter two are involved in wound and inflammation reactions.

Yet in spite of the widespread occurrence of cell death in the development of animals

and plants, the biochemistry of dying cells has hardly been investigated at all; it is not even mentioned in most textbooks of biochemistry. But it cannot be ignored. Haberlandt showed many years ago that, in plants, damaged and dying cells could produce hormones; and it now seems likely that hormones are produced as a consequence of cell death during normal development. The evidence discussed above is strongly in favour of the normal production of auxin occurring in this way; it seems probable that autolysing cells can also be a source of cytokinins and ethylene; it is possible, too, that gibberellins are produced as a consequence of cytolysis. Nevertheless, while cytolysing cells may be important sources of these hormones, they may not be the only sites of their production. Cytokinins, for example, are apparently made by living cells in root tips.

The reader will have observed that the evidence for the production of hormones as a consequence of cell death is in many cases indirect and circumstantial. But hypotheses are guesses as to what might be the case rather than statements of fact. The alternative to the hypothesis that hormones are produced as a consequence of cell death is the hypothesis that hormones are synthesized by living cells. The latter, however implicitly it is accepted, cannot be taken for granted. Only further research can establish the relative contributions of living and autolysing cells to hormone production in plants.

XI. SUMMARY

1. Although much is known about the effects of plant hormones and their role in the control of growth and differentiation, little is known about the way in which hormone production is itself controlled or about the cellular sites of hormone synthesis. The literature on hormone production is discussed in this review in an attempt to shed some light on these problems.

2. The natural auxin of plants, indol-3yl-acetic acid (IAA) is produced by a wide variety of living organisms. In animals, fungi and bacteria it is formed as a minor by-product of tryptophan degradation. The pathways of its production involve either the transamination or the decarboxylation of tryptophan. The transaminase route is the more important.

3. In higher plants auxin is also produced as a minor breakdown product of tryptophan, largely via transamination. In some species decarboxylation may occur but is of minor importance. Tryptophan can also be degraded by spontaneous reaction with oxidation products of certain phenols.

4. The unspecific nature of the enzymes involved in IAA production and the probable importance of spontaneous, non-enzymic reactions in the degradation of tryptophan make it unlikely that auxin production from tryptophan can be regulated with any precision at the enzymic level. The limiting factor for auxin production is the availability of tryptophan, which in most cells is present in insufficient quantities for its degradation to occur to a significant extent. Tryptophan levels are, however, considerably elevated in cells in which net protein breakdown is taking place as a result of autolysis.

5. An indole compound, glucobrassicin, occurs in *Brassica* and a number of other

genera. It breaks down readily to form a variety of products including indole acetonitrile, which can give rise to IAA. There is, however, no evidence to indicate that glucobrassicin is a precursor of auxin *in vivo*.

6. Conjugates of IAA, e.g. IAA-aspartic acid and IAA-glucose, are formed when IAA is supplied in unphysiologically high amounts to plant tissues. These and other IAA conjugates occur naturally in developing seeds and fruits. There is no persuasive evidence for the natural occurrence of IAA-protein complexes.

7. Tissues autolysing during prolonged extraction with ether produce IAA from tryptophan released by proteolysis. IAA is produced in considerable quantities by autolysing tissues *in vitro*.

8. During the senescence of leaves proteolysis results in elevated levels of tryptophan. Large amounts of auxin are produced by senescent leaves.

9. Coleoptile tips have a vicarious auxin economy which depends on a supply of IAA, IAA esters and other compounds closely related to IAA from the seed. These move acropetally in the xylem and accumulate at the coleoptile tip. The production of auxin in coleoptile tips involves the hydrolysis of IAA esters and the conversion of labile, as yet unidentified compounds, to IAA. There is no evidence for the *de novo* synthesis of IAA in coleoptiles.

10. Practically all the other sites of auxin production are sites of both meristematic activity and cell death. The production of auxin in developing anthers and fertilized ovaries takes place in the regressing nutritive tissues (tapetum, nucellus, endosperm) as the cells break down. In shoot tips, developing leaves, secondarily thickening stems, roots and developing fruits auxin is produced as a consequence of vascular differentiation; the differentiation of xylem cells and most fibres involves a complete autolysis of the cell contents; the differentiation of sieve tubes involves a partial autolysis. There is no evidence that meristematic cells produce auxin.

11. The lysis and digestion of cells infected with fungi and bacteria results in elevated tryptophan levels and the production of auxin. Viral infections reduce the levels of tryptophan and are associated with reduced levels of auxin.

12. Crown-gall tissues produce auxin. It is suggested that the crown-gall disease may involve at any given time the death of a minority of the cells which produce auxin and other hormones as they autolyse; the other cells grow and divide in response to these hormones.

13. Auxin is produced in soils, particularly those rich in decaying organic matter, by micro-organisms. This environmental auxin may be important for the growth of roots.

14. There is no convincing evidence that auxin is a hormone in non-vascular plants. The induction of rhizoids in liverworts by low concentrations of auxin can be explained as a response to environmental auxin.

15. Abscisic acid is synthesized from mevalonic acid in living cells. It is possible that under certain circumstances, abscisic acid or closely related compounds are formed by the oxidation of carotenoids.

16. The sites of gibberellin production are sites of cell death. It is possible that precursors of gibberellins, such as kaurene, are oxidized to gibberellins when cells die.

17. Cytokinins are present in transfer-RNA (tRNA) of animals, fungi, bacteria and

higher plants. They are probably formed in plants by the hydrolysis of tRNA in autolysing cells. There is evidence that they are also formed in living cells in root tips.

18. Ethylene is produced in senescent, dying or damaged cells by the breakdown of methionine.

19. It was shown many years ago that wounded and damaged cells produced substances which stimulate cell division. It now seems likely that the production of wound hormones and the normal production of hormones as a consequence of cell death are two aspects of the same phenomenon. Wounded cells can produce auxin, gibberellins, cytokinins and ethylene.

20. The control of hormone production in living cells is a biochemical problem which remains unsolved. The control of production of hormones formed as a consequence of cell death depends on the control of cell death itself. Cell death is controlled by hormones which are themselves produced as a consequence of cell death.

21. In spite of the fact that dying cells are present in all vascular plants, in all wounded and infected tissues, in certain differentiating tissues in animals, in cancerous tumours and in developing animal embryos, the biochemistry of cell death is a subject which has been almost completely ignored. Dying cells are an important source of hormones in plants; some of the many substances released by dying cells may also be of physiological significance in animals.

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