CHAPTER 6

Growth Is Affected

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I. Introduction

One of the most striking characteristics of higher plants and animals is the extraordinary way in which all of their functional parts fall into a coherent and flexible, but definitely limited pattern. In the normal organ-
ism, morphogenetic laws are strictly obeyed and processes concerned in metabolism, growth, cellular differentiation, and organogenesis are precisely regulated. These processes start and stop in harmony to give the organism certain proportions that remain constant from one generation to the next.

The normal plant may be looked upon as a precisely balanced complex of interrelated reactions that are in a state of dynamic equilibrium. Any deviation in this complex of reactions will tend to disrupt the balanced system and may lead to atypical, abnormal, or pathological growth. It is not surprising, therefore, that growth patterns of plants may be readily modified by external environmental conditions such as heat, cold, light, and humidity, by nutritional deficiencies and excesses, by radiation, by changes in genetic constitution, as well as by pathogenic organisms of the most diverse types. The borderline between the normal and the abnormal in plants is, therefore, often quite indistinct and may represent merely quantitative changes which are evidenced either by a harmonious but somewhat exaggerated manifestation of the normal developmental processes or, as is more commonly found to be true, by an arrest or inhibition of the processes concerned with growth and development. At other times, orderly qualitative changes result which in their most interesting form reveal growth and developmental potentialities in a plant far beyond any realized in the past history of the species. In the most extreme instances, plant cells undergo far-reaching qualitative changes and may, as a result, become permanently modified into new cell types in which continued unregulated and unorganized growth, rather than differentiation, characterizes the behavior of the affected cell.

Of particular concern to the present discussion is the influence that parasites of many different kinds exert on the growth and development of a parasitized plant.

Gäumann (1954) has indicated that organisms are pathogenic only if they are toxigenic. Although this sweeping generalization probably contains a large element of truth, little is actually known yet about the physiological and chemical mechanisms underlying disease. It is nevertheless true that the growth of systemically diseased plants is generally inhibited. Even in those instances in which the disease is very mild and symptoms are largely masked (latent virus infections of potato, masked strains of tobacco mosaic virus), a statistically significant inhibition of growth of the host plant is found to occur. More often, pronounced stunting is a characteristic manifestation of disease in plants. This may result either from systemic infection, or from the diffusion of specific chemical compounds elaborated by a pathogen, which itself remains localized in
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the host. These chemical substances fall essentially into two categories: (1) enzymes; (2) soluble metabolites, many of which are capable of reproducing perfectly the toxic manifestations of disease and some of which have been characterized chemically. (See further discussion in Chapter 13 (Volume II of this treatise).

Of far greater biological interest than the substances which act in such an unsophisticated way to damage, kill, or otherwise inhibit growth of the cells of the host, are those substances that stimulate cells to excessive growth.

The regulation of growth, differentiation, and organ formation in higher plants appears to result, among other things, from a very precisely balanced series of growth-regulating substances or hormones, on the one hand, and from inhibitory systems or other compensatory mechanisms that control the synthesis and response of plant cells to such biologically active substances, on the other. Certain metabolites elaborated by pathogens appear to be quite similar or identical in their physiological action to growth-regulating hormones found to occur naturally in a plant. When these are produced in excess by the pathogen during the course of infection, growth responses occur—in a host—that simulate those found following the artificial application of excessive amounts of such growth-promoting substances. At other times, the chemical stimuli produced by the pathogen are quite different from any thus far recognized in the normal plant and, being different, tend to divert the normal growth pattern of the plant into new and unusual directions. Such morphogenetic stimuli appear to be of many different types. They are commonly highly specific in their action and regularly induce unusual growth responses in an affected plant. It must be recognized, however, that whatever form the growth abnormality takes, the potentialities for this form must have been present in the cells of the host. These growth potentialities are simply called into activity by the inciting stimulus. Thus, the morphogenetic stimulus produced by the pathogen as well as the protoplasmic substrate of the host upon which the stimulus acts are critical in determining the type of growth that results.

Finally, the metabolite elaborated by the pathogen may not itself be a hormone or a morphogenetic stimulus of the type referred to above which directly influences the growth pattern of the host. It may instead affect specifically the regulatory mechanism of the host cells by eliminating either temporarily or permanently those cellular systems that are concerned with the regulation of growth or, alternatively, it may activate within the affected cell growth-substance-synthesizing systems whose
products are concerned specifically with growth and developmental processes. In these instances, it is the affected cell itself that elaborates growth-promoting substances—in greater than regulatory amounts—in response to a specific stimulus transmitted by a pathogen.

II. HARMONIOUS CHANGES INVOLVING EXAGGERATED GROWTH RESPONSES

A. Generalized Stimulation

1. Bakanae Effect

Intensified manifestations of normal developmental potentialities have been recorded in a number of plant species following infection with a variety of fungi, many of which are obligate parasites. The common houseleek, Sempervivum hirtum, grows as a rosette. The leaves of this plant are broadly ovate in form and are about twice as long as they are broad. Following infection with the rust Endophyllum sempervivi the appearance of the leaves is altered. They grow strongly in length and may be seven times as long as they are broad. As a result, the leaves assume a linear shape. Infected leaves stand erect and are much paler in color than are their normal counterparts.

Elongation of the internodes with a resulting significant increase in size appears to be a characteristic response of certain plants to infection by specific pathogenic fungi. Following infection of one of the spurges, Euphorbia cyparissias, by the aecial stage of the rust Uromyces pisi, the stem of the host elongates greatly. The distances between successive leaves in healthy Euphorbia plants are about 0.5 mm. while those of infected plants are 2-3 mm. The foliage leaves of the normal plant are thin, flexible, and about twelve times as long as they are broad. In diseased specimens, the leaves are thick, brittle, and only about two to three times as long as they are broad. Infected plants have an etiolated appearance. Similar responses have been observed in Vaccinium vitis-idaea infected with the teleutospore stage of Melampsora goeppertiana, in sugar cane with the downy mildew Sclerospora sacchari, and in Bromus erectus with the smut fungus Ustilago hypodyes. Alterations produced on the shoots of the periwinkles Vinca herbacea, Vinca major, and Vinca minor by the uredospore stage of the rust Puccinia vincae and on the shoots of Cirsium arvense by the teleutospore stage of Puccinia suaveolens are also very similar to those described above for Euphorbia. The stems of the infected plants become much elongated, while the leaves are shorter, broader, yellow in color, and brittle when compared with the normal. Frequently, the shoots blossom prematurely and the flowers are more or less abortive. When, for example, Primula clusiana and P. minima are infected with Uromyces primulae integri-
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folae, not only do the rosette leaves elongate but the flowers open in the autumn of the same year rather than in the following spring as happens in the normal uninfece specimens.

Pilet (1952, 1953) studied the auxin content of healthy leaves of Sempervivum sp. infected with Endophyllum sempervivi as well as of healthy and diseased leaves of Euphorbia cyparissias infected with Uromyces pisi in an attempt to account—on a physiological level—for elongation of the stem as a result of infection. Parasitized leaves of both plant species revealed a much higher auxin content than normal. On the basis of these observations, Pilet (1953) suggested (1) that the causal fungus elaborates auxins, (2) that the increased auxin level found in the host is produced by the host in response to the parasite, or (3) that the parasite produces something which activates precursors of auxins in the host cell and which in turn are converted into auxin. It has not yet been shown, however, that the application of auxin to normal plants results in an elongation of leaves and internodes comparable to that obtained after infection by specific rust fungi. Brian (1957) has, therefore, suggested that the increased auxin found in the leaves and shoots of such plants may be coincidental rather than causal since quite different metabolic products of fungi, the gibberellins, have been shown to be responsible for stem elongation in the bakanae disease of rice as well as in many other plant species when these substances are applied artificially.

The bakanae or foolish seedling, disease of rice is the best understood of this type of disease. It has been studied by Japanese pathologists for many years. A comprehensive review of this disease has recently been presented by Stowe and Yamaki (1957). The bakanae disease, which is caused by Fusarium moniliforme (imperfect stage) or Gibberella fujikuroi (perfect stage), is widely distributed and found in most rice-growing regions of the world. Fusarium moniliforme has a large host range, is soil-borne, and attacks the roots and basal portions of the stem not only of rice plants but of maize, cotton, sugar cane, and other plant species as well. In the case of rice, a browning of the tissue usually occurs at the site of infection, the leaves tend to yellow and curl inward and, in many instances, growth of affected seedlings is arrested. However, some of the affected plants grow more rapidly than do healthy ones and are conspicuous in the field because of their height and etiolated appearance. The dry weight of the elongated seedlings was found to be significantly greater than that of the healthy plants. This overgrowth effect—occurring naturally—has been reported only in rice, although artificial inoculation has resulted in increased growth in maize, barley, sugar cane, sorghum, millet, wheat, and oats.
Kurosawa (1926) showed that the overgrowth or bakanae effect could be reproduced in rice seedlings by treating them with cell-free culture filtrates on which the causal fungus had grown. These filtrates reproduced all of the characteristic manifestations of the bakanae disease such as lengthening of the internodes and leaves, chlorosis (except under conditions of nutrient excess), and reduced tillering. This pioneer work of Kurosawa was followed by the isolation and chemical characterization of several biologically active substances. Two powerful growth-promoting substances, which were given the trivial names of gibberellin A and B, were isolated in crystalline form by Yabuta and Sumiki (1938), and Yabuta and Hayasi (1939a, b, 1940). It was found later that gibberellin A is converted to B by warming in dilute acid at 50°–70° C. Boiling under acid conditions converted both substances into a biologically inactive compound named gibberic acid. A third biologically active substance, gibberellin C, was isolated by Yabuta et al. (1941a) from acid-treated gibberellin A. The nature of the gibberellin skeleton was established by Yabuta et al. (1941b). It was demonstrated in these studies that gibberene obtained by selenium dehydrogenation of gibberellin A, B, or gibberic acid is a fluorene derivative.

Another quite distinct but chemically related, biologically active compound was isolated by American workers headed by F. H. Stodola and by British investigators under P. W. Brian. This substance was named gibberellin X by the Americans and gibberellic acid by the British group. The latter name is now commonly used for this compound. Although gibberellic acid, whose empirical formula is C_{19}H_{22}O_{6}, resembles gibberellin A in biological activity and degradation products, it differs from this compound in infrared spectra, optical rotation, in certain derivatives, as well as in the empirical formula. The basic skeleton of gibberellic acid, like gibberellin A, was found to be gibberene, which Mulholland and Ward (1954) identified as 1,7-dimethylfluorene. This led Cross et al. (1956) to suggest the tentative structure for gibberellic acid shown in Fig. 1. It is reasonably certain that the formula shown in Fig. 1 is essentially correct, although the exact points of attachment of the lactone grouping to the cyclohexenol ring of gibberellic acid are not known as yet.

Recently, Takahashi et al. (1955) reexamined gibberellin A and found this compound to be essentially homogeneous by countercurrent distribution, paper and partition chromatography. Esterification followed by chromatography on alumina led to the isolation of three methyl esters which were named gibberellin A_1, A_2, A_3. Stodola et al. (1957) believe that their gibberellin A is identical with A_1. Gibberellic acid was shown to be identical with A_3. Gibberellin A_2 has been isolated only in Japan.
but Stodola (1956) reports its corrected empirical formula as $C_{19}H_{26}O_6$. Thus, the existence of three distinct gibberellins appears to be established.

Evidence is now available suggesting that the gibberellins elaborated by the fungus *Gibberella fujikuroi* correspond closely (in their biological activity) to growth-regulating compounds found to occur naturally in higher plants. West and Phinney (1957) have isolated—but have not yet identified chemically—an ether-extractable substance from wild cucumber seeds which, like the gibberellins of fungus origin, promotes the active growth of dwarf mutants of maize. Since these genetic dwarfs appear to be due to single-gene defects, they may be explained as resulting from blocks in the biosynthetic pathway leading to the formation of a natural gibberellin-like substance. The gibberellins appear to substitute in the plant for the missing product of that reaction. Very recently MacMillan and Suter (1958) have obtained high yields of gibberellin $A_7$ from the seeds of runner bean plants. The isolation and characterization of gibberellin $A_7$ from a higher plant indicate further that this compound, in all probability, participates directly in the growth-regulating system of higher plants and, therefore, represents a new type of endogenous growth regulator.

![Gibberellic acid](image)

**FIG. 1.** Gibberellic acid.

The gibberellins of fungal origin have been applied to a large number of different plant species and have produced a variety of responses. The most consistent and striking response is a marked elongation of plant stems. In one survey of 42 different plant species belonging to many different families, only 3, white pine, gladiolus, and onion, failed to respond with stem elongation. In many instances, not only do the stems grow longer but also they are proportionately thicker. Gibberellin-treated oak and maple trees are not only taller but have a diameter more than twice as great as that of untreated control specimens. Brian and Hemming (1955) have reported remarkable stimulation of dwarf varieties of
peas, broad beans, and French bean by gibberellic acid. The treated dwarfs and untreated normal plants grew equally well.

In the bakanae disease of rice as well as in certain related diseases described earlier in this discussion, infected plants that reached maturity flowered earlier than did normal plants of the same variety. With the availability of pure gibberellic acid for experimental purposes, Lang (1956) found that a biennial variety of *Hyoscyamus* treated with that substance bolted and flowered in the first year. In a later study, Lang (1957) found that in annual varieties of *Hyoscyamus*, *Silene*, and *Samolus* the long-day requirement for flowering could also be replaced by gibberellic acid. Wittwer *et al.* (1957) found that gibberellic acid-treated lettuce bolted and flowered under photoperiodically adverse conditions. These same workers reported the gibberellins to be 500 times more effective than indoleacetic acid in inducing parthenocarpy in tomatoes.

Reduced fruit yields are characteristic of the bakanae disease. Hayashi *et al.* (1953) found that the gibberellins reduced rice grain production by 32% although the yield of straw was increased by 14%.

Stowe and Yamaki (1957) suggest that the one property common to the gibberellins is their capacity to remove certain limitations on cell enlargement, while incidentally releasing other responses. Despite this similarity to the auxins, the gibberellins are not auxins but appear to depend for their activity on the presence of auxins. The biological activity of these substances has been attributed (Brian, 1958) to the neutralization of a growth-inhibitory system which normally limits growth.

Certain other plant diseases give indication that infection interferes with the synthesis or utilization of gibberellin-like substances. Carrot plants infected with the tomato big-bud virus of California, as well as with a related virus found to occur naturally in carrots in the State of Washington, were shown by Kunkel (1951) to bolt and flower prematurely. These findings suggest that virus infection may increase the synthesis of gibberellin-like substances by the plant. On the other hand, in the rosette diseases, as exemplified by peach and peanut rosette, infection may interfere with the normal synthesis or utilization of these biologically active substances by the plant. The stunting effects, but not other symptoms associated with aster yellows, corn stunt, and wound tumor diseases, have in fact been reversed with gibberellic acid (Marromorosch, 1957).

**B. Growth Changes in Leaves and Petioles**

1. **Growth Movements**

Nastic responses refer to changes in position of a bilaterally symmetrical organ as a result of differential growth. Epinasty of leaf petioles
has been found by Wellman (1941) to be a characteristic early symptom of the *Fusarium* wilt disease of tomato plants. Hunger (1901) and Smith (1920) found it to be associated with Granville wilt, while Grieve (1941) recorded this condition as a primary symptom in roses affected with the rose wilt virus. Pronounced epinasty has also been produced experimentally by Locke *et al.* (1938) following inoculation of tomato plants with a highly virulent strain of the crown gall bacterium. Hypo­nasty, on the other hand, has been observed in plants infected with *Erwinia phytophthora*.

Grieve investigated the question of epinasty of the leaf petiole as it occurs in the Granville wilt disease (1936, 1939, 1940). In this instance, the growth response was found to be an irreversible one and the invasion of one lateral trace by the bacteria was sufficient to induce it. Grieve reported that a growth substance which appeared to be indoleacetic acid was elaborated by the bacteria in culture and that this substance induced a characteristic epinastic response when applied to tomato plants. However, virulent and avirulent cultures of the bacterium produced approximately equal amounts of the growth-promoting substance. Furthermore, no significant difference between the growth-substance content of comparable control and infected stem segments could be detected. Studies of the growth-substance distribution in upper and lower halves of reflex­ing petioles, on the other hand, showed a significantly greater concent­ration in the upper halves. In normal petioles, the concentration of growth substance was found to be greatest in the lower halves of the petioles. Grieve (1939) reached no definite conclusion as to how the redistribution of growth substance in the basal part of the petiole is accomplished. He pointed out (Grieve, 1943), however, that the balance of the normal growth-controlling mechanism at the base of the petiole is very delicate, as evidenced by the fact that ethylene, in one part in ten million parts of ambient air, as well as very minute amounts of growth substance from bacterial cultures will disturb it. Grieve considers that even a small stimulus from the invading organisms can initiate a chain of reactions that leads to a redistribution of growth substance with a resulting nastic response.

More recently, Dimond and Waggoner (1953) made a detailed inves­tigation of the cause of epinastic symptoms in *Fusarium* wilt of tomato plants. From these studies it was concluded that ethylene is responsible for the characteristic growth response. This conclusion was based on the finding that ethylene is produced in culture by the causal agent of this disease, *Fusarium oxysporum* f. *lycopersici*. Ethylene production by infected tomato plants was demonstrated, moreover, by confining such plants with healthy indicator plants. Under these conditions, epinastic responses developed to a greater degree than when the indicator plants
were confined with healthy tomato plants. In these studies, ethyl alcohol, which is capable of causing epinasty in tomato and which is produced by the causal fungus in culture as well as in the infected host, was also considered a possible cause of the growth response. This compound was ruled out, however, because the amounts produced by the fungus in the hosts were insufficient to account for the observed epinastic responses.

Another characteristic response—involving growth movements—which is associated with certain disease conditions is concerned with the upright growth habit of leaves. This is one of the most characteristic symptoms of the aster yellows disease in many different plant species. Leaves on a normal aster plant take approximately a horizontal position in relation to the main axis of the stem. In virus-infected plants, on the other hand, the petioles elongate and the leaves assume an extremely upright habit of growth which approximately parallels the main axis of the plant. It is this upright habit of growth that suggested the name "rabbit ears" for lettuce plants infected with the aster yellows virus.

Kunkel (1954) has shown that twigs of peach trees infected with peach yellows virus characteristically assume an upright growth habit. In this instance, the plants can be cured of the disease by thermal treatment. After cure, the new growth of such twigs again takes a normal position with respect to the main stem. This change necessitates a change in the direction of growth of the terminal bud.

2. Curling and Distortion

The curling and distortion of leaves are characteristic of many diseases. Needless to say, such well known maladies as curly top of sugar beet, peach leaf curl, and leaf roll of potatoes have been assigned their trivial names because curling of leaves is the most conspicuous symptom of the disease. The most extreme form of leaf rolling is found in the so-called scroll galls produced by certain insects. Under the influence of specific insects, the leaves curl lengthwise to form tightly rolled scrolls in which the insects live.

Peach leaf curl caused by *Taphrina deformans* is perhaps the most studied of this type of disease. The causal fungus commonly infects very young leaves which soon become either quite red or paler in color than are the normal leaves. Such infected leaves soon become curled and puckered, increase greatly in thickness, and have a firm consistency. The host cells which are in contact with the invading fungus are stimulated to abnormal activity. This may involve isolated regions of a leaf or in extreme instances most of the leaf. Such cells increase in size and number and produce marked changes in the form and structure of the leaf. Loss of chlorophyll is almost complete in the stimulated cells. The
increase in size of the cells on either side of the midrib results in a puckering of the leaf. At the same time, the leaves tend to curl and become concave on the lower side. The cells of the palisade parenchyma respond much more actively to the stimulus of the fungus than do the subjacent cells of the spongy parenchyma, thus producing the curling. Link et al. (1937) have reported that a substance having the properties of an auxin and which was presumably indoleacetic acid was extractable from *Taphrina deformans* culture filtrates. The role, if any, that this substance plays in the development of the disease picture as described above is as yet not clear. It is nevertheless true that auxins are capable of stimulating cell enlargement of the type described above in parenchymatous cells of many different plant species.

3. *Frenching and Shoestringing*

Frenching, a well-known deformity of tobacco, occurs in most tobacco-growing regions of the world. This condition is, in an advanced stage, characterized by a cessation of terminal bud and stem growth, and by a reticular type of chlorosis in the slowly expanding new leaves. This chlorosis may disappear as the young leaves develop and become strap-shaped (sword and string) as a result of the failure of the leaf lamina to expand. As apical dominance in such diseased plants is lost, the axillary buds develop and an unusually large number of leaves—which may be as high as 300—appears on a plant. These leaves assume an upright growth habit and are commonly sword- or string-shaped. Such plants have the appearance of a rosette or, in extreme instances, of a witches’ broom. Root growth is also somewhat inhibited, although the effect on the leaves appears to be the most characteristic feature of the disease.

The extent to which frenching modifies the morphology of a leaf can be seen from some figures that are given by Wolf (1935). Normal tobacco leaves average 55 cm. in length and 30 cm. in width. The corresponding measurements for sword leaves and string leaves are 40 cm. × 10 cm., and 27 cm. × 1.8 cm., respectively. Histological studies show, moreover, that the diseased leaves are two to three times as thick as normal leaves. This increase in thickness is due to an enlargement of cells of all the leaf tissue except cells of the vascular system. Schweizer (1933) has indicated that the xylem is markedly reduced in development in frenched leaves. In mature string leaves, the parenchyma remains quite juvenile with little or no evidence of dorsiventrality.

Although the cause of frenching is not clearly established, it is now believed to be a toxicity disease rather than the result of a nutritional deficiency. No parasitic organism of any kind has yet been implicated
in this condition. McMurtrey (1932) described briefly the similarity of
frenching symptoms to those of thallium toxicity in tobacco. Spencer
(1935) found that one part of soil obtained from a field in which
frenching occurred mixed with 2,000 parts of sand produced typical
frenching in the test plants. Thus, a toxic factor, effective in very low
concentrations, was present in frenching soils. Spencer (1935) tested 33
different elements on tobacco and found, as had McMurtrey, that only
thallium at a concentration of 5 p.p.m. or less produced chlorosis, strap-
shaped leaves and other symptoms characteristic of frenching. Later,
Spencer and Lavin (1939) indicated that frenching and thallium toxicity
are probably two distinct physiological conditions.

Steinberg (1947, 1950) has suggested that frenching of tobacco is
caused by the action of diffusates from the presumably nonpathogenic
soil bacterium *Bacillus cereus*. The effectiveness of *B. cereus* diffusates
in eliciting typical frenching symptoms in tobacco varied with the kind
and quantity of peptone used and the concentration of inorganic nitrogen
in the test medium.

*Bacillus cereus* is a widely distributed soil microorganism. The pro-
gressive development and type of symptom produced by it in tobacco
(in aseptic culture) largely parallel those that appear in plants subject
to this abnormality under field conditions. Moreover, rhizosphere and
rhizoplane counts of this organism increased by 65% and 200%, respec-
tively, when frenching occurred in the field. The nature of the toxic
substance is not yet known.

Steinberg (1952) has shown that slight excesses of certain amino
acids caused production of symptoms resembling frenching in tobacco
seedlings. Frenching symptoms were, however, limited to the natural
isomers of alloisoleucine and isoleucine in tobacco and to these com-
pounds and leucine in *Nicotiana rustica*. Alloisoleucine was most effec-
tive in both species and as little as 2 to 8 p.p.m. resulted in chlorosis and
strapping of leaves in tobacco. Leucine, which was ineffective when
applied to tobacco, was more effective than isoleucine in *N. rustica*.
Analytical studies indicated, moreover, that frenching was found to be
accompanied by a marked increase in isoleucine and other free amino
acids in the leaf lamina of field-grown plants (Steinberg *et al*., 1950).
The absence of free amino acids in frenching soil and the marked
increase of free isoleucine in frenched leaves indicate that *Bacillus cereus*
toxin and isoleucine are not identical. The conclusion was therefore
drawn that the accumulation of excessive quantities of free amino acids
in the strapped leaves was a probable chemical factor involved in the
production of the morphological symptoms in the plant. The stages sug-
gested by Steinberg (1952) leading to abnormal growth in frenching of
field tobacco were, therefore, as follows: bacterial soil toxin $\rightarrow$ receptor $\rightarrow$ excessive accumulation of isoleucine and other free amino acids in the leaves $\rightarrow$ frenching.

A condition resembling frenching may also be caused by certain strains of the tobacco mosaic and cucumber mosaic viruses or a mixture of these viruses, as well as by a gene mutation. Kunkel (1954) has reported an extreme instance of shoestringing caused in tomato plants by a mixture of tobacco mosaic virus and the virus of carrot yellows of Texas. Leaf-blade development in this instance was completely suppressed. Just how these viruses suppress or prevent leaf-blade growth is not understood.

The so-called "wiry" tomato plants described by Lesley and Lesley (1928) are not unlike those which result from the virus diseases of the shoestring type reported above. Wiry plants appear, however, to result from genic mutation. This condition is recessive to the normal and the plants are completely sterile. The leaves of these plants are variable in shape but have a strong tendency toward reduction of the leaf lamina and in extreme instances the leaves consist merely of a tapering midrib.

C. Growth Changes in Stems and Branches

1. Organs Arising in Unusual Places

   a. Adventitious Roots. The development of adventitious roots on stems of plants has been reported to be a characteristic host response associated with certain bacterial, fungal, and viral diseases.

   A number of investigators (Bryan, 1915; Grieve, 1936, 1940; Hunger, 1901; Smith, 1914, 1920) have described this phenomenon following infection of tomato and certain other hosts with *Pseudomonas solanacearum*. Smith (1914, 1920) reported it to be associated with the Grand Rapids disease of tomato which is caused by *Corynebacterium michiganense*. Adventitious root formation was found to occur on tomato (Locke et al., 1938) and *Kalanchoe* (Price and Gainor, 1954) following inoculation of those host species with the crown gall bacterium. It has also been observed by Wellman (1941) and Dimond and Waggoner (1953) in tomato plants diseased with *Fusarium* wilt. Certain viruses, such as the cranberry false blossom virus in tomato as well as that implicated etiologically in the sereh disease of sugar cane, characteristically stimulate root formation in the stems of their respective hosts.

   Grieve (1936, 1940) studied the development of adventitious root formation in tomato plants infected with *Pseudomonas solanacearum*. This investigator found that the new roots arise commonly over a span of several internodes along the path of the primary bundle except in
those instances in which the disease progresses with great rapidity, in 
which case these adventitious structures do not develop. Histological 
examination of transverse and longitudinal sections of infected plant 
 stems demonstrated that adventitious roots usually develop in regions 
where large primary bundles are affected. The initiation of root pri-
mordia often precedes the advancing bacteria, indicating action at a 
distance. Grieve applied indoleacetic acid to the stems of tomato plants 
and found that the roots initiated were similar to those resulting from 
infection. This finding suggested that the bacteria are inducing root 
formation either directly as the result of the production of a growth-
promoting substance, or indirectly through their interference with the 
metabolism of the plant. In attempting to distinguish between these two 
possibilities, Grieve found that \textit{Pseudomonas solanacearum} produces in-
doleacetic acid from tryptophan in culture. However, virulent and aviru-
lent strains produce approximately equal amounts of this substance. The 
possibility that the bacteria produce an auxin in the xylem by acting on 
naturally occurring or artificially introduced tryptophan was examined 
but no evidence for such production was obtained. No difference in 
auxin concentration could be detected, moreover, between healthy and 
diseased plants by bioassay methods. Furthermore, adventitious roots 
could be produced by cutting or blocking the bundles. These findings 
led Grieve to question whether the bacteria induce the formation of 
adventitious roots directly through the elaboration of indoleacetic acid. 
He suggested (Grieve, 1943) that the production of these structures 
more likely results from disturbances of normal auxin transport in the 
plant as a result of mechanical blocking of the vessels by the bacteria.

Price and Gainor (1954) reported a striking correlation between 
adventitious root development and the number of leaves present on a 
\textit{Kalanchoe} plant inoculated with crown gall. These workers suggested 
that a substance responsible for stimulation of adventitious roots is organ-
ized in leaves and is transported downward to the site of the tumor. 
That the biologically active substance is not indoleacetic acid was indi-
cated by the finding that 2\% of this substance in lanolin—applied to 
stumps of petioles above the points of inoculation—did not stimulate 
the development of adventitious roots in \textit{Kalanchoe daigremontiana}.

De Ropp (1947a) has shown, moreover, that a powerful root-stimulat-
ing substance is elaborated by sterile crown gall tumor cells. In crown 
gall it appears likely, therefore, that the stimulus for adventitious root 
formation originates in the cells of the host rather than directly from 
the causal bacteria.

The course of \textit{Fusarium} wilt of tomato is not unlike that of the 
bacterial wilt of this host as described above. Wellman (1941) reported
adventitious roots associated with this fungus disease. Since epinasty of the leaf petioles often appears on plants showing adventitious root formation, Dimond and Waggoner (1953) have suggested that in the case of *Fusarium* wilt, adventitious root formation, like epinasty, may result from the production of ethylene either by the causal fungus or by the cells of the host as a result of the interaction of the host and the pathogen.

b. *Adventitious Shoots.* The witches'-broom virus in potato causes the infected plant to produce numerous buds at the nodes in the above-ground stems of potato plants. Long slender stolons that resemble aerial roots but which are covered with trichomes develop from these adventitious buds. Aster yellows virus and carrot yellows virus from Texas, on the other hand, stimulate the production of small aerial tubers in the axils of the leaves of potato plants.

An extreme example of adventitious shoot formation was reported about 100 years ago by von Martius. This worker described an unusual plant that possessed a mania for forming innumerable leaves and shoots. This species has been appropriately named *Begonia phyllomaniaca*. Plantlets develop spontaneously in incredible numbers from the superficial cell layers of the leaf lamina, petioles, and stems. Erwin Smith (1920) studied phyllomania and believed it to be conditional on shock such as is encountered during the repotting of plants. Smith indicated, moreover, that the cells of leaves and internodes are susceptible to such shock only during a relatively brief period of meristematic growth. The adventitious shoots do not arise from preformed buds but develop from totipotent cells at the base of the trichomes and especially from botryose glands which are found in great abundance in young stems and leaves of this species. These embryo plants develop a vascular system of their own but the vast majority never succeed in connecting this with the vascular system of the plant. They must, therefore, be considered not as branches but rather as independent organisms.

2. *Witches'-Brooms*

Witches'-brooms, or "hexenbesens," are closely grouped, much branched structures commonly found to occur on a number of different species of trees and shrubs. The stimulus necessary for their formation is supplied in different plant species by pathogens of the most diverse types. Fungi of the genus *Taphrina* and various rust fungi are effective in inducing witches'-broom, while distinct virus species have been shown to cause the formation of such an abnormality in alfalfa, potato, peach, and the black locust. Bos (1957) has recently presented a detailed account of the virus-induced witches'-broom. An eriophyid mite belong-
ing to the genus *Aceria*, followed by a powdery mildew fungus, is said to be involved in the development of branch knot so commonly found on the hackberry.

Typical witches'-brooms caused by the fungi appear to live a more or less independent life and act as parasites on the plants from which they are derived. In accordance with their independent existence, witches'-brooms tend to break away from the correlations of the parent plant. Instead of branching out horizontally, the brooms stand as more or less erect clusters of branches. A normal dorsiventral symmetry is thus changed to a radial symmetry. Witches'-brooms as a rule do not produce flowers, indicating further a breakaway from the correlations of the parent plant. Heinricher (1915) has shown, moreover, that a twig of a witches'-broom caused by *Taphrina cerasi* grafted to a healthy sweet cherry tree develops again into a typical witches'-broom. The independence of these structures is further shown in an impaired periodicity. The vegetative buds found on the brooms of the sweet cherry, for example, open several weeks earlier than do those present on healthy branches (Schellenberg, 1915). Gäumann (1946) has pointed out that this premature unfolding of the buds is probably associated with the fact that the shoots and buds comprising the broom never achieve a true winter dormancy since the pathogen never becomes entirely quiescent. This incomplete winter dormancy may result in a winter killing of the first year twigs present on a broom.

The witches'-broom of the silver fir caused by *Melampsorella caryophyllacearum* is typical of this peculiar type of growth. The primary infection is said to occur in the young bark of branches surrounding buds. In the spring when the buds develop, the fungus mycelium grows into the epidermis of the developing shoot and penetrates the cortex so that by fall a slight swelling of the shoot axis is found. During the following year, an overgrowth of considerable size may be formed and buds embedded in this growth develop to produce the characteristic deformation. The twigs of this particular type of broom are found to develop in whorls. They are short, thick, soft, and pliable. This results from the fact that the cortical parenchyma is spongy and the wood is not well developed. The buds on the broom open earlier in the spring than do those present on a healthy twig, while the leaves found on the diseased specimens remain short, are yellowish in color, and fall off when a year old. The leaves present on a normal twig are, on the other hand, long, straight, dark green on the upper side, and commonly remain in position 5 or more years. The longevity of the diseased twigs themselves is limited and they die within a few years, giving rise to the dry, bristling brooms characteristically found on the silver fir.
The witches'-broom-like effects that occur in certain plant species following virus infection appear to result from the excessive stimulation and development of secondary shoots. This condition is not accompanied by swellings or overgrowths characteristic of many of the fungus-induced brooms.

Brian (1957) has suggested that the diseases characterized by excessive branching may well be due to auxin deficiency rather than to auxin excess. Lacey (1948) has, in fact, shown that cultures of Corynebacterium fascians, the causal agent in leafy gall development on certain plants, are capable of rapidly destroying indoleacetic acid and other auxins present in plant tissues.

D. Growth Changes in Floral Organs

1. Alterations of Floral Parts

Under the stimulus of pathogens, the sepals, petals, stamens, or pistils of a flower may be transformed into structures that are very different in appearance from those found normally. Kerner von Marilaun (1891) has reported that double flowers are produced in Valerianella carinata, the common corn salad, as a result of infestation by a mite. This doubling results from a retrograde alteration of the stamens into a whorl of petals. These petals, under the stimulus of the mite, enlarge to more than fifty times their original size and finally appear as fleshy lobes which fuse with one another into a disc. The greatly enlarged lobes bend backward and are concave on the lower side. It is in the cavities thus formed that the gall mites live.

In the capitula of certain of the milfoils, Achillea millefolium and A. nana, the peripheral ray florets and the central tubular ones become leaf-like in appearance and assume remarkable forms as a result of mite infestation. A capitulum is often subdivided into several stalked sub-capitula, while the flowers are altered into green funnel-shaped structures with jagged mouths and into small flat-lobed green foliage leaves, while short green scale-like leaflets, which represent modified stamens, develop from the midribs of these leaves. Sometimes, however, the changes in growth pattern following mite injury are not so extreme as the two instances cited above might suggest. In Veronica saxatilis, for example, the development of numerous hairs on the rachis of the raceme, the pedicels, and the bracts appears to be the only characteristic host response. Hairs are not present in the corresponding normal structures of this plant species.

Doubling of the flowers of the alpine rose, Rhododendron ferrugineum, has been reported by Kerner von Marilaun (1891) to result
from gnat infestation. In this instance the stamens and carpels are transformed into red petals. Since flowers of this species normally have 10 stamens and 5 carpels, there should be only 15 red petals in the center of each but there are often two or three times that many present. It thus appears that not only metamorphosis but multiplication takes place.

According to Tschirch (1890), the aphid *Astegopteryx stryacophila* induces remarkable changes in young flowers of *Styrax benzoin*. The calyx, corolla, and androecium are transformed into large abnormal leaves that form bag-like pockets. The pistils, however, appear to be unaffected in this instance.

Metamorphoses of floral parts of higher plants are also brought about by certain species of fungi. The transformation of stamens into petals commonly follows infection of *Viola sylvestris* by *Puccinia violae*. Similarly, *Peronospora violacea* has been reported to encourage stamen primordia in the flowers of *Knautia arvensis* to develop into petal-like structures. An extreme instance of phyllody is found when flowers of the Japanese plum are infected with a rust of the species *Caema makinoi*. In this instance all of the floral parts are transformed into foliage leaves. In the alder the bracts of the pistillate flowers are changed by *Taphrina alni-incanae* (*Amentorum*) into greatly elongated purple-red spatulate lobes which are twisted and bent.

Von Tubeuf (1895) has reported that when *Albugo candida* infects the inflorescences of the radish, the ovary, calyx, and corolla enlarge and the androecium assumes a leaf-like appearance. Stamens may become green and leaf-like in the downy mildew disease of *Pennisetum glaucum* and other grasses. Thus, this disease, which is caused by *Sclerospora graminicola*, is popularly known as green ear. In the case of head smut of maize, caused by *Sorosporium reilianum*, the whole staminate head or the ear may become a leafy structure.

In the United States, there are at least three different viruses, the aster yellows virus, the cranberry false blossom virus, and tomato big bud virus, that produce gigantism in the floral organs and more particularly in the sepals and calyxes of tomato and certain other solanaceous plants. The tomato big bud virus appears to affect the sepals rather specifically. These structures enlarge greatly under the influence of this virus and fuse to form huge bladder-like growths that conceal the inner parts of the flower. The stamens and pistils do not appear to be greatly affected. The aster yellows virus and the cranberry false blossom virus cause symptoms in the flower trusses of tomato that are almost identical with those caused by the big bud virus. Although these three viruses cause the sepals of certain of the solanaceous plants to enlarge greatly, they
do not have this effect on sepals of species of certain other families. They do not, for example, cause gigantism in the sepals of the flower trusses of the periwinkle, *Vinca rosea*, but they bring about a virescence in the petals, stamens, and styles. There is, in this instance, a retrograde development of floral parts into foliage leaves. It is, therefore, clear that the type of symptom produced depends as much on the species of plant in which the virus multiplies as on the nature of the virus.

The aster yellows, cranberry false blossom, and carrot yellows virus of Texas stimulate the formation of adventitious buds in the stigmas of flowers of a number of different hosts. Such buds may give rise to either flowers or to leafy stems which in turn may bear flowers.

Bos (1957) has recently attempted to interpret the development of the flower and its component parts on the basis of the phenomenon of antholysis accompanying virus infections in *Crotalaria* and certain other hosts. This investigator has suggested, on the basis of the homology of the reproductive and vegetative parts of a plant and their morphogenetic development, that the sexual and vegetative activities in the plant are mutually antagonistic. A growing point can develop only into an inflorescence or into a vegetative shoot. During flower initiation the vegetative characters are suppressed and sexual characters prevail. The sexual characters remain suppressed, on the other hand, during vegetative growth. From the manner of appearance of antholysis in virus-infected *Crotalaria* plants, Bos has concluded that flower induction is stopped suddenly and that subsequent development of the floral parts proceeds exclusively in a vegetative manner. The ultimate result obtained depends upon the stage of development of the primordia in the bud at the time of suppression of flower induction by the virus. Thus, flowers initiated in succession, produce a series of flowers showing increasing antholysis. These—in reverse order—clearly show a macroscopically recognizable picture of the morphogenesis of the flower and of its component parts. Therefore, according to Bos, antholysis supports the theory that the flower must be regarded as a modified leafy branch.

2. Overcoming of the Normally Arrested Development of Floral Parts

The anther smut, *Ustilago violacea*, produces its spores only on the anthers of certain hosts. When this fungus infects the female flowers of *Melandrium album* or *M. dioicum*, the stamens, which are normally arrested in their development and are present only in a rudimentary form in the pistillate flowers, grow to full size but when mature are filled with smut spores instead of pollen.

A similar process is said to occur when pistillate flowers of *Knautia*...
arvensis and K. sylvatica are infected with Ustilago scabiosae. The nature of the morphogenetic stimulus which overcomes the arrested development of male sex organs in female flowers is still unknown.

E. Modification in Fruiting Structures

Modification of the fruiting bodies of higher plants occurs quite commonly as a result of infection. Such descriptive names as little peach, bladder plum, phony peach, etc., have been applied to conditions of this type. Hypertrophied fruits are commonly produced in certain species of the genus Prunus as a result of infection by Taphrina pruni. In this instance, the tissue of the diseased ovary is stimulated to growth, but not in the same way as in the normal fruit. The resulting body is flattened on two sides, is brittle, yellow in color, and much longer than the normal fruit. The seed within is abortive and a hollow space is left in its place. These hypertrophied growths, which are commonly called "bladder plums," fall from the trees at the end of May and are said to be eaten in certain areas. A somewhat similar condition is found when Taphrina aurea infects the pistillate flowers of the poplar. This fungus, like the one described above, stimulates the growth of the ovaries with the resulting development of golden yellow capsules that are more than twice the normal size. The smut fungus Ustilago zaeae also stimulates growth of the tissue of the pistillate flowers of maize. As a result, the grains are replaced by irregular cushion-like structures with a diameter of up to 7 cm. The resulting growths contain more auxin than do normal tissues. Ustilago zaeae, moreover, produces an auxin in culture (Moulton, 1942) which was identified as indoleacetic acid by Wolf (1952). The fruit size may be greatly dwarfed as in the case of two virus diseases, little peach and phony peach, or the fruits may assume an asymmetrical shape as is found characteristically in the xyloporosis disease of oranges—which is also of viral origin.

The seed capsules of Datura stramonium normally bear numerous conspicuous spines. When plants of this species are infected with the severe etch virus, spine formation may be completely inhibited (Kunkel, 1944). The aster yellows virus, on the other hand, causes a bursting of the abnormal fruits produced by Cajophora lateritia plants infected with this agent. The seed-like structures present in these fruits are green in color and have been transformed, under the influence of the virus, into short stems which bear leaves.

The entire inflorescence may, in certain disease conditions, be altered to quite a different type. The rust fungus, Aecidium esculentum, is said to cause the normal inflorescence in Acacia to change from a head to a spike. Similarly, the stinking smut fungus, Tilletia tritici, may change the
growth pattern of the head of the club types of wheat to the elongated or "vulgare" type.

Individual floral parts or even entire organs may be replaced by new structures in certain diseases. *Claviceps purpurea* invades and destroys certain of the ovaries of rye and other grasses. In place of the seeds, elongated dark purple sclerotia are produced. These are composed of dense aggregates of fungus mycelium.

In sheep sorrel infected with *Ustilago oxalidis* the seeds are replaced by the spores of the pathogen. In this instance, the spores are forcibly expelled from the seed capsule as if true seeds were present.

**F. Fasciation**

A condition known as fasciation can be classified between that group of growth abnormalities described above (involving harmonious changes in growth pattern) and the amorphous changes to be considered later in this discussion. Fasciation is a morphological term that has been used to describe a series of abnormal growth phenomena resulting from many different causes, any of which result at the morphological level in a flattening of the main axis of the plant. Although this ribbon-like expansion of the stem is often the most striking feature of this condition, all parts of the plant may be affected. Fasciation often results in alterations in the arrangement of foliar and floral structures. White (1948) points out that, when fasciation occurs, the early seedling growing stages are normal. As the plant develops, however, the growing point becomes broader, and the unregulated, distorted tissue growth results in significant increases in weight and volume of plant tissue. The apical growing region becomes linear and comb-like in some instances or develops numerous growing points, producing a witches'-broom effect. In still other instances, the growing points may be coiled and resemble a ram's horn or they may be highly distorted into a grotesque tangle of coils. Fasciations are widespread, both geographically and taxonomically. Examples have been recorded in 102 families of vascular plants. Fasciations found in certain plant species such as the common cockscomb, *Celosia cristata*, as well as the cristates in the cacti are highly prized by gardeners.

Fasciations have been classified on morphological grounds—based on such physical features as form, color, and anatomical structure. Linear, bifurcated, multi-radiate, and ring or annular types have been listed (de Vries, 1909–1910; White, 1948). This condition has also been classified from a causal standpoint. In this type of classification the conditions and agencies necessary to produce the character as well as the modifying factors that affect its development are considered. White (1948) has placed these essentially into five categories (1) fasciations which breed
true and which in crosses with the normal obey genetic laws so that their
genetic basis is known; (2) noninherited forms in which the fasciation is
due to environmental causes and the character is not reproduced in the
selfed seed; (3) fasciations that occur spontaneously and which have
been propagated vegetatively but in which neither the initial cause nor
the question as to whether the condition is transmitted through the seed
is known; (4) fasciations which are induced artificially by known pro-
cedures; (5) fasciations which have been imperfectly investigated.

Fasciations resulting from modifications of the normal gene com-
plement, and which breed true, have been known for centuries in such
plants as *Celosia* as well as in the mummy or crown type of garden pea.
Gregor Mendel (1866) found fasciation in the mummy pea to be reces-
sive in the *F*₁ when crossed with normal varieties. Such *F*₁'s showed a
ratio of 3 normals to 1 fasciate in the *F*₂. A gene-fasciated race in *Nicot-
tiana tabacum* when crossed with nonfasciated strains, on the other
hand, gives 1:2:1 *F*₂ ratios. In other instances, very complicated results
were obtained, showing the effects of modifying factors and giving rise
to nonfasciated segregates which carry the fasciated gene. Fasciation is
not correlated with changes in chromosome numbers but it may produce
meiotic irregularities.

Aside from gene mutation, fasciation has been reported to result
from environmental effects such as frost, pressure, alteration of food and
water relationships, pruning, mutilation, as well as by more specific
etiological agencies such as a bacterium, *Corynebacterium fascians*, and
X-irradiation.

There seems little doubt but that nutritional changes due to correla-
tive disturbances in growth-substance relationships play a role in fascia-
tion. Bloch (1938) has reported that this condition can in certain
instances be produced experimentally by treating plants with auxin-
containing pastes. On the other hand, high doses of X-rays have been
shown to inactivate auxins and yet disturbances caused by them also
result in fasciation. The effects of X-irradiation on auxin metabolism in
the growing plant are obviously complex. These nongenetic types of
fasciation are not transmitted through the seed but may, in some in-
stances, be propagated asexually. Both inherited and noninherited types
of fasciations which are phenotypically indistinguishable have been
described for at least 3 different plant species.

Orland E. White believes that the basic cause of fasciation is a dis-
turbed metabolism involving excessive nutriment which mobilizes energy
that must be used. This energy, once accumulated, goes, according to
White (1948), into growth and becomes "wildly" expended in abnormal
and unpredictable tissue production. Heslop-Harrison (1952) points out,
however, that these conclusions are descriptive rather than truly explanatory since excessive nutrition in itself cannot account for the abnormal element in fasciation. He believes, rather, that a proximate cause of these abnormalities is probably to be found in maldistribution of the auxins in the plant. Jones (1935) and Orland White (1948) have suggested that fasciation bears an analogy to animal cancer. In the opinion of the writer, however, this condition does not represent a true tumor at all but might better be placed in that category of teratological abnormalities known as monstrosities.

III. Amorphous Changes in Growth Pattern

Amorphous changes involving either the temporary or permanent loss of typical organization are found to occur very commonly in plants. These overgrowths range from somewhat exaggerated but self-limiting wound-healing responses at the one extreme, to rapidly growing non-self-limiting tumors which have no characteristic size or structure, at the other.

A. Self-Limiting Overgrowths

1. Intumescences

Intumescences, which are among the simplest and most innocuous of this type of growth, are found to occur most commonly on leaves but are also found on stems and fruits of plants. These blister-like pustules usually result from the abnormal elongation of groups of cells with or without increased cell division. In a typical leaf intumescence found in many plant species, the palisade parenchyma cells elongate considerably in localized areas to give rise to the pustules. Intumescences have been found to be produced experimentally in a number of ways. No pathogens, however, appear to be concerned in their development. Sorauer (1886) believed them to result from an excess of moisture in the air and soil. Harvey (1918) produced intumescences artificially on the undersurface of cabbage leaves by subjecting such leaves to slight freezing. Smith (1920), on the other hand, induced blister-like pustules in cauliflower leaves with the use of such irritating chemicals as formic and acetic acids. Wolf (1918) has shown that intumescences may be produced in cabbage leaves by means of a sandblast and this worker has indicated that such overgrowths found naturally in the field result from sand driven against the leaves by wind. More recently, La Rue (1933a, b, 1935) was able to reproduce perfectly the intumescences which arise under conditions of high humidity on the leaves of poplar with the use of low concentrations of indoleacetic acid. Much larger self-limiting over-
growths were produced artificially by Brown and Gardner (1936) on bean plants with the use of higher concentrations of that substance. It is not unlikely that certain of the burls as well as the overgrowths that sometimes arise at graft unions result from a hormonal imbalance of this type.

2. Galls

Localized overgrowths in which the host cells are stimulated to excessive growth by pathogens are known as galls. In these instances, the continued growth of the host cells is dependent upon continued stimulation by the pathogen. There are unusually large numbers of distinct overgrowths of this type to be found in plants of which only a few representative examples have been selected for discussion.

a. Insect Galls. Among the most interesting types of self-limiting growth abnormalities found in plants are those that result from the activity of certain of the gall-forming insects. The whole subject of insect galls as reviewed in detail by Küster (1911), Ross and Hedicke (1927), and more recently by Felt (1940) suggests that many of these highly specialized overgrowths represent beautiful examples of dependent differentiation.

Insect galls may result either from a mechanical or chemical stimulus. Stem swellings on roses caused by the closely placed spiral galleries of the rose stem girdler, *Agrilus viridis*, are believed to be an example of the former type. Chemical stimulation, however, is probably far more important than mechanical irritation in the production of many insect galls, although the chemical stimulus may in some instances be supplemented by directive feeding of the insect. An examination of the literature leaves the unmistakable impression that highly specific morphogenetic stimuli, of chemical nature, and elaborated by insects, are capable of initiating, stimulating, and directing most precisely the development and differentiation of plant cells. There is no question about the fact that the morphological form that a gall assumes depends upon the nature of the pathogenic insect. This is evidenced by the fact that the same host species or even the same organ of the same plant attacked by different but closely related insect species produces morphologically very different galls. Figure 2 illustrates this point and shows drawings of 4 galls of quite different morphology induced on leaves of the California white oak by 4 closely related species of cynipids. The morphology of the gall is so specific that it is considered by some to be a more reliable criterion for distinguishing between closely related species of insects than are the morphological characters of the adult insects. The morphology of the gall does not, moreover, appear to be a function of the part
GROWTH IS AFFECTED

of the plant from which it arises. Currant galls of the oak, which are found on both flower stalks and leaves, have similar morphological structure at both points of origin. The same insect species may, moreover, produce the same gall type on different species of plants. The sawfly, *Micronematus gallicola*, produces bright red galls of similar morphology on four different species of willow. Findings such as those reported above suggest in the strongest possible manner that a large number of highly specific chemical substances are elaborated by insects and that these substances have specific morphogenetic effects on the cells and tissues of a plant. However, only moderate success has thus far been achieved in establishing experimentally the truth of this view. That a morphogenetic stimulus is elaborated by certain insects is suggested by the fact that the Norway spruce aphid, *Adelges abietis*, attaches itself firmly by its beak to the scale of a fir bud and can directly influence only a few cells of the young shoot. Nevertheless, thousands of cells on this shoot are affected and soon begin to assume an altered form and are stimulated to growth, indicating that a diffusible stimulus is active. Plumb (1953) has, in fact, induced the formation of this gall by injecting a glycerol extract of the salivary glands of the insect into the bases of spruce buds. Beijerinck, as early as 1888, killed the eggs of a sawfly with

![Fig. 2. Four morphologically distinct galls produced on leaves of the California white oak by four closely related species of insects. (Drawings by R. J. Mandlebaum.)](image-url)
a hot needle immediately after they had been deposited in the host and found that the galls developed normally. Beijerinck called the biologically active materials “growth enzymes,” and in 1897 applied this concept to the development of form in general. Parr (1940) showed that the gall-forming coccid, *Asterolecanium variolosum*, stimulates the host tissue to both hyperplastic and hypertrophic activity. He demonstrated further that the effect of the salivary secretion of this homopteron continues to stimulate plant cells even after the insect itself is gone. It was possible to reproduce galls similar to those produced by the insect by injecting glycerol extracts of the salivary glands. When the salivary extracts were heated to 60°C they did not produce galls, indicating that a heat-labile, enzyme-like substance was involved in gall formation.

Rössig (1904) investigated various organs of gall wasps and believed that he could trace the site of formation of the gall-forming substances to the Malpighian vessels. Similar findings were reported by Triggerson (1914) for the cynipid which produces the oak-hedgehog gall. Lewis and Walton (1947) reported some very interesting histological and cytological findings dealing with a biologically active substance believed to be involved in the growth and differentiation of cell development of the cone gall of witchhazel. This gall is produced by the aphid species *Hormaphis hamamelis* Fitch. In this instance the stem mother inserts her stylets into and between the cells of an immature leaf and injects very minute droplets of a substance secreted by glands opening into the stylar canal. This stinging process is not to be confused with feeding and the sting substance is quite different in its nature from substances found in the salivary secretions. When about 150 stings have been made in a small circular area by the stem mother, the cells in this region at first become etiolated. Almost immediately thereafter, cells receiving injected sting material dedifferentiate, undergo rapid mitotic divisions, and then redifferentiate into gall cells rather than typical leaf cells.

The injected sting material consists of a ground substance in which minute crystalloids are embedded. These crystalloids show a reddish purple cast in such stains as gentian violet, Congo red, and acid fuchsin. They are capable of passing readily from cell to cell as well as of entering cells when they are deposited by the insect in the intracellular spaces. Whether the injection is inter- or intracellular, the end result is the entry of the crystalloids into the nucleolus. There they may fuse to form a large crystalloid which again breaks up into smaller ones as mitosis begins. During nuclear division, the crystalloids are distributed to the daughter nuclei where they are again found in the nucleolus. Since these bodies are incapable of self-propagation and since they are apparently used up during growth of the cells, fresh sting material must be injected
repeatedly by the stem mother during the entire growth of the gall. It would indeed be interesting to know the chemical nature of the “crystalloids” since they possess some very unusual regulatory properties.*

J. P. Martin (1942) induced galls on three-month-old sugar cane plants by injecting extracts obtained from macerated leaf hoppers of the species Draculacephala mollipes. These findings again appear to implicate hormone-like substances in the initiation and development of insect galls. Anders (1958) reported that he was able to reproduce the swellings associated with the Phylloxera disease by applying to grape plants secretions obtained from the aphid responsible for this condition. A similar type of swelling was obtained when certain amino acids, used in the same proportions found in the insect secretions, were applied to grape roots.

The larva of the moth, Gnorimoschema gallowolidaginis, induces an elliptical monothalmous gall on the stem of the Solidago host. The larva in this instance burrows into the terminal bud of the plant and then down the stem to a point 2 cm. below the growing point, where it eats away the central tissues and induces the formation of a simple spindle-shaped gall. The gall stimulus, according to Beck (1954), appears to be associated with a silky substance secreted by the feeding larva. This silky material induces anatomical changes in normal stems similar to those found in the galls. The stimulus is, however, short-lived and uniform and continued deposition of the silky substance over the surface of the larval chamber appears necessary for the formation of typical galls.

Boysen-Jensen (1952) studied, with the use of rather ingenious methods, the development of a midge (Mikiofo fagi) gall on beech leaves. Evidently the formation of the gall in this instance is caused by growth substances given off by the larva. These as yet uncharacterized substances produce cell enlargement and cell division but not organized growth. It was, therefore, suggested that cell enlargement and cell division are regulated by the larva which moves rapidly about the gall chamber and secretes the growth-promoting substances in definite places, thereby making the gall assume its special form. According to this interpretation, the growth-promoting substance does not have special organizing properties but the shape of the gall is dependent upon the distribution of a rather nonspecific type of growth substance by the larva. The growth substances are, in other words, tools which are used

* After this paper had been submitted to the editors, a comprehensive account of the studies of Lewis and Walton (1958) appeared. In that investigation the diagnostic crystalloids were found to be Feulgen-positive, and it was concluded that the gall results from the activities of a virus. On the basis of the evidence presented, this conclusion is, in the opinion of the author, unwarranted.
by the gall larva to model a gall from the cells of a beech leaf. While such a mechanism as that proposed by Boysen-Jensen may satisfactorily explain the formation of certain simple galls, it is difficult to see how it would explain the development of the more intricate structures the cells of which are highly differentiated.

The distinguishing feature of insect galls in general, and the more highly developed cynipid galls in particular, is the determinate growth of these structures. Bloch (1954) has suggested that an insect gall is almost comparable—in its "determinate" growth—to a leaf or a fruit. These galls are constant in form and size and possess their own polarity and symmetry. Although the cells of these galls dedifferentiate as a result of the initial stimulus, they again redifferentiate into an orderly rearrangement of cells and cell layers which possess a degree of differentiation that is never below that of the host. A fascinating field lies open here for exploration at the morphogenetic and biochemical levels.

b. Root Nodules. The root nodules found to arise on many species of leguminous plants as a result of infection by bacteria of the genus *Rhizobium* are, like the insect galls, highly organized and specialized structures. These nodules, unlike most of the overgrowths described here, are by and large highly beneficial to the host because of the role that they play in nitrogen fixation.

The typical root nodule is composed of four histologically well defined regions. The outermost tissue, or nodular cortex, consists of several layers of parenchymatous cells that originate from the nodular meristem found immediately below the cortex at the distal end of the nodule. The meristem is conspicuous and is composed of small, compact, rapidly dividing cells. The cortex and meristem are normally free of bacteria. The provascular tissue of the nodule arises as a result of radial divisions and differentiation of cells—at the periphery of the inner infected cells and the nodule cortex—at the time when the nodule is still meristematic. These differentiate later into a typical vascular bundle which consists of xylem, phloem fibers, sieve tubes, and companion cells enclosed in parenchymatous tissue and surrounded by an endodermis. The vascular system of the nodule is connected with that of the host and is functional. The central region of the nodule is composed of two types of parenchymatous cells, infected and noninfected, and is commonly known as the bacteroid zone.

Essentially, two types of nodules have been recognized on the basis of their origin. The more common or exogenous type arises, except for the vascular linkages, from the cortical parenchyma of the root. The second or endogenous type is found far less frequently and is composed of cells that arise from proliferation of the pericycle.
The information presented above concerning structure clearly indicates that the nodule is not a shapeless mass of cells but instead represents a highly organized growth. This information, in turn, permits a consideration of the possible mechanisms involved in the growth and development of the nodule.

The infecting bacteria commonly enter the host through root hairs, although in certain aquatic plant species that do not possess root hairs they may and commonly do enter through epidermal cells. Prior to invasion of the host, the bacteria form a small colony close to the tip of the root hair. Under these conditions, the hair curls very characteristically and assumes the form of a "shepherd's crook." As early as 1900, Hiltner found that sterile filtered bacterial extracts, when applied to root hairs, produced a comparable type of deformation. Similar results were reported by Thornton (1936) and Thornton and Nicol (1936) who showed that such filtrates stimulated the production, growth in length, and characteristic deformation of the root hairs. Later, Thornton (1947) found that pure β-indoleacetic acid, when applied to root hairs, elicited a curling similar to that found prior to invasion by the bacteria. Probably, therefore, the characteristic curling of the root hair is the result of the production of an auxin by the bacteria although this point has not yet been fully established. Wilson (1940) points out, for example, that the characteristic curling of the hair is not easily explained on the basis of a growth hormone present in the sterile culture filtrate. He states that one would not expect to find differential growth rates under such experimental conditions since the concentration of the hormone should be the same on all sides of the roots.

After the bacteria enter a hair, they align themselves into hyphalike zoogloea infection thread. The thread penetrates to the base of the hair and then into and directly through the subjacent cortical cells where it branches. As the zoogloeaal strands migrate through the cortical cells, they become encased in a sheath composed of cellulose-like material, which on the basis of cytochemical studies is said to be composed of the same material as is the wall of the host cell. This has led to the suggestion (McCoy, 1932) that the sheath is deposited by the host cells rather than by the zoogloeaal strand and that it serves as a defense mechanism against the invading bacteria. Of particular interest to the present discussion is the fact that proliferation of the root cells and subsequent development of the nodule do not occur until the bacteria are released from the infection thread. Release may be achieved essentially in the following ways (Allen and Allen, 1954). The bacteria may be discharged from the tip of the thread as the thread migrates. They may be released as a result of the rupture of unsheathed globular protuberances that are
commonly found to be present in infection threads or they may be liberated following stresses accompanying cell enlargement and cell division. Wipf and Cooper (1940) have suggested, on the basis of cytological studies, that invasion of the disomatic cells by the bacteria is essential for the release of the bacteria from the infection thread as well as for cellular growth terminating in nodule formation.

Once the free bacteria reach the cytoplasm, they tend to migrate to the peripheral regions of the cell and begin to multiply. The host cells containing the organisms, as well as the adjacent uninfected cells, undergo rapid division and subsequent enlargement. Infected cells may increase eightfold in size. Since neighboring uninfected cells are also stimulated to grow, it has been suggested by McCoy (1929) that the bacteria elaborate a diffusible substance which stimulates cell enlargement and cell division. Ultimately, many of the cells in the heart of the nodule become filled with bacteria and the rods found in nodules of certain, but not all, plant species become highly pleomorphic. It is in this so-called bacteroid zone of the nodule that symbiotic nitrogen fixation takes place. This zone also contains four highly interesting pigments, one of which, leghemoglobin, is closely related chemically to blood hemoglobin. The question of the occurrence of the pigments in nodules and their possible role in nitrogen fixation has been reviewed in detail by Allen and Allen (1950, 1954).

Attempts have been made throughout the years to explain in physiological terms the mechanism involved in the development of the nodule. As early as 1912 Molliard reported that he had produced tuberizations on roots of pea plants by treating the roots with cell-free filtrates upon which the rhizobia had grown. It was concluded from these experiments that the bacteria elaborated some biologically active, growth-promoting substance which brought about changes in the root similar to those found during actual infection. Thimann (1936, 1939) examined this question further and postulated the following series of events after the bacteria had penetrated the root. During the course of their metabolism within the host cells the bacteria elaborate a small amount of auxin and certain other substances. The auxin causes an enlargement of the cells in which it is produced but, being diffusible, enters the pericycle and stimulates growth and division, thus giving rise to the early stages of a lateral root initial. In the presence of continued auxin production by the bacteria, the potential lateral root is prevented from elongating. Instead, the cells increase isodiametrically in size. Uninfected cells are stimulated to division by auxin diffusing from the infected cells. In this way, Thimann considers, "a shapeless mass of parenchymatous tissue is produced which is essentially a lateral root prevented from elongating."

Many investigators do not believe, however, that a nodule is simply
a modified lateral root. Fred et al. (1932) have discussed the evidence for and against this concept in detail and conclude that the nodule is not a modified lateral root "for it has no central cylinder, root cap, or epidermis. Furthermore, it does not digest its way out from the cortex of the main root but remains covered with a considerable layer of cortical parenchyma."

Nevertheless, growing nodules are active auxin-producing centers and that substance plays an important role in the growth of the nodule (Link and Eggers, 1940). Thimann (1936, 1939) has shown, moreover, that auxin production in the nodule roughly parallels the growth of the nodule.

Chen (1938), Georgi and Beguin (1939), Link (1937), Rasnizina (1938), and Thimann (1939) have demonstrated that in vitro the root-nodule bacteria produce considerable quantities of auxin from tryptophan, yeast extract, and peptones present in an otherwise suitable culture medium. Hunt (1951) found, moreover, that free tryptophan was present in the nodules. An evaluation of these findings is complicated, however, by the observation made by Chen (1938) and Georgi and Beguin (1939). These workers found that both effective and ineffective strains of rhizobia produced auxin from tryptophan and in some instances the ineffective strains appeared to be more efficient growth-substance producers than were the effective strains.

While it has been possible to produce overgrowths on the roots of certain leguminous plants by applying growth substances of the auxin type, it nevertheless is true that histologically and anatomically these artificially induced growths do not bear the slightest resemblance to nodules produced under the stimulus of the bacteria. Allen and Allen (1954) point out that the nodule is not a shapeless mass of cells but is a well organized structure. Thus, while there appears to be no conflict on the question of the increased growth-substance content of the nodule, a suitable explanation regarding the role that such a substance plays in directing the organization of the nodule is not yet at hand.

c. Root Knot. Root knot, which is caused by species of nematodes belonging to the genus Meloidogyne, is a destructive disease in many cultivated and wild plants. The overgrowths produced on the roots may appear as small scattered tubercles or as extensive swellings which may reach diameters up to 2 in. and involve almost the entire root system. Severely infected roots have a rough, clubbed appearance not unlike that encountered in the club root disease of the crucifers. The mature female nematode is embedded in the plant tissue while the eggs are commonly found to be clinging to the sides of the roots. When the eggs hatch, the young larvae may again penetrate young roots just below the growing
point. Once embedded in the tissue, the larva begins to feed and almost at once stimulates the growth of the host cells which leads to the development of the typical knot. The gall, in this instance, is composed of relatively few, greatly hypertrophied cells together with cells showing hyperplasia. Often, 6 to 20 nuclei are present in the enlarged cells although Němec (1910) has reported more than 500 in a single cell of *Vitis gongylodes*. The multinucleate giant cells appear early in gall development. They are rich in storage materials and serve to nourish the larvae in much the same manner as do the "nutritive cell" layers found in certain insect galls. It would appear likely that at least two distinct stimuli, one of which is concerned with cell enlargement and which involves only a few cells and the other with cell division, are involved in the development of this type of overgrowth.

Dropkin (1954) has recently shown a high positive correlation to exist between gall area and numbers of larvae in a gall. He therefore concluded that the response of the root to the presence of the nematode is a local one and that the size of the gall ultimately produced is a function of the amount of stimulation provided by each larva present in the overgrowth. Here, then, is another example of a self-limiting growth, the development of which is dependent upon continued stimulation by the pathogen.

d. Clubroot. The typical overgrowth produced on the roots of cabbage by *Plasmodiophora brassicae* is spindle-shaped, thick in the middle, and tapering gradually toward the ends. Such a club is a morphological unit and is commonly the result of a single primary infection. Sometimes, however, the swellings resulting from two or more primary infections fuse to form a compound club which is more irregular in outline than those resulting from a single primary infection. Cunningham (1914) has recognized different types of hypertrophy in different species of susceptible hosts. In cabbage, a complete clubbing of the main roots and of the lateral roots may occur, while in *Sisymbrium altissimum* the lateral roots are commonly free of distortion. In *Sisymbrium officinale* and *Erysimum cheiranthoides*, on the other hand, clubs develop on the lateral roots but not on the main root. Both lateral roots and the main root are affected in *Lepidium sativum*. In this instance, however, club-free rootlets are found above the diseased portions. In the radish, *Raphanus sativus*, clubs occur as tumors on the root.

As a result of the overgrowths, infected roots fail to absorb nutrients from the soil and are often unable to transport food and water taken up by healthy roots. This functional failure of the roots results in the starvation and, ultimately, severe stunting of affected plants. Heavily infected seedlings are usually killed before they reach maturity.
The spread of the disease-producing agent from points of primary infection is accomplished in cabbage through a direct penetration of the host tissue by the infecting plasmodia, which then grows and divides repeatedly. According to Kunkel (1918), infection by penetration may be divided into four parts: (1) primary infection of the cortical tissue and penetration to the cambium, (2) infection of the cambium in all directions from the point of original penetration, (3) passage of the plasmodia out of the cambium into the cortex and in from the cambium toward the xylem region, and (4) infection of the medullary rays.

As the plasmodia pass through the tissues, some of them become established within cells, while others continue to penetrate into the deeper regions. Although no noticeable effect is observed in a cell if infection is temporary, stimulation leading to abnormal cellular growth and division results if the infection of a cell is permanent. The period of infection continues up to the time that the host plant stops growing or dies. The infected cells of a club are distributed in small groups throughout the diseased tissue and do not lie adjacent to each other. The stimulus resulting in abnormal cellular growth appears to travel in advance of the infection. This can be clearly seen when infection is established in the medullary rays. The ray cells—in the proximity of diseased cells—increase considerably in size. This increase in size as well as in number of cells tends to split the central cylinder of an infected root. The mass of parasitic protoplasm in a given volume of diseased tissue is, according to Kunkel (1918), remarkably constant in different clubs. This indicates that the amount of cell growth in this disease, as in the case of root knot, is dependent upon the mass of parasitic protoplasm in the diseased tissue. Clubroot can be considered to be a malignant disease in the sense that this malady may and frequently does kill plants that are affected by it. However, there is no evidence that the affected cells themselves have become permanently altered or that such cells are capable of continued abnormal growth in the absence of the pathogen.

At this point in the discussion we have reached the borderline between the self-limiting growths and the true or non-self-limiting tumors.

B. Non-Self-Limiting Tumors

Tumors of a non-self-limiting and transplantable type may develop in many plant species just as they do in many kinds of animals. In diseases of this type the new growth or tumor is composed of altered, randomly proliferating cells that reproduce true to type and against the growth of which there is no control mechanism in the host. The diseased cells acquire autonomy which permits them to direct their own activities
largely irrespective of the laws that govern very precisely the growth of all normal cells in a higher plant or animal. The nature of this acquired autonomy is fundamental and constitutes the ultimate basis of the tumor problem.

Since the tumor problem is basically a cellular problem and since the fundamental similarity of cells and cellular processes in plants and animals is commonly recognized, certain plant tumors have for many years provided interesting and unique experimental material for studying the metabolic processes that underlie the tumorous state. Certain of the early workers, particularly C. O. Jensen, who is generally considered to be the father of modern experimental cancer research, and Erwin F. Smith, studied on a comparative basis the crown gall disease of plants and malignant animal tumors and they found that these two types of growth have much in common. In comparing plant and animal tumors it must be remembered, however, that there are certain developmental and functional characteristics commonly used in the differentiation of animal tumors that are more or less restricted to animals and cannot, therefore, be carried over and applied to plant tumors. These have been dealt with in detail by White and Braun (1942), and Black (1949) and will not be considered further here. The most essential characteristic of being able to grow independently of any morphogenetic restraint, upon which all other diagnostic features must ultimately depend, is, however, equally capable of expression in neoplasia of all higher organisms since it is a characteristic property of the cell itself.

While it is generally true that the more malignant a tumor cell is the greater is its capacity for growth, it is equally true that a tumor cell is not characterized primarily by its rate of growth. Certain normal cell types may grow and divide at far faster rates than do most tumor cells. Regenerating liver cells, for example, grow much more rapidly than do hepatoma cells present in that organ. Similarly, the meristematic cells at the apex of a rapidly developing root or shoot grow and divide at considerably faster rates than do plant tumor cells. Thus, it is not rapid growth but autonomous growth that characterizes the tumor cell.

Autonomy of neoplastic growth is not, however, a fixed and unvarying character but it has many gradations. At the one extreme are found the slow-growing benign tumors that remain localized in the host. At the other extreme are the most malignant cancers that grow rapidly, infiltrate neighboring tissues, metastasize, and kill. Theoretically, autonomy of tumor cells requires within them something newly activated and distinctive, something that urges such cells to continued abnormal and unregulated growth. One of the difficulties encountered in attempting to define the nature of autonomous growth has resulted from the fact that
some of the most diverse agencies known to biologists are capable of accomplishing this condition. Such factors as radiant energy, irritation, carcinogenic chemicals, microorganisms, and viruses have all been shown to be more or less effective tumor producers in certain animals and plants. The effectiveness of these agencies in eliciting tumor formation appears to be determined in large part by the hereditary constitution of the host. These agencies, with the exception of certain of the viruses, are concerned only with the inception of the tumors and play no role in the continued unregulated growth of a tumor cell. It is therefore necessary to distinguish between the proximate cause or causes concerned in tumor initiation and the continuing cause or causes responsible for tumor development.

Three non-self-limiting diseases of plants, each of which has a different and quite distinct initiating cause, have been studied fairly extensively. These are: (1) the crown gall disease in which an as yet uncharacterized tumor-inducing principle elaborated by a specific bacterium regularly converts normal plant cells to tumor cells, (2) Black's wound tumor disease which is of known viral etiology, and (3) Kostoff's tumors which have a genetical basis and which commonly arise as a result of such a nonspecific stimulus as irritation in certain interspecific hybrids within the genus Nicotiana. These are pictured in Fig. 3. There are other true tumorous diseases of plants that have not as yet been extensively studied. Most interesting among these is White's spruce tumor (White and Millington, 1954a, b; Reinert and White, 1956), the etiology of which has not as yet been established.

Although the crown gall, wound tumor, and the genetic tumors have quite different and distinct initiating causes, there is every indication that the ultimate continuing cause is similar at a physiological level in the three types of growth.

The development of an acceptable concept designed to explain the continued abnormal growth of a tumor cell in the absence of any recognizable infective agent (except in the case of the virus-induced tumors) represents a very real challenge to students of abnormal growth. Such a concept must not only explain the underlying basis for autonomous growth but it must also account for the morphological, histological, and cytological peculiarities that characterize the tumorous state.

Plant tumors may assume a variety of growth patterns. These range from slowly growing benign to rapidly growing malignant, and from completely unorganized to highly organized teratomatous growths. All may be produced in certain test systems under precisely defined experimental conditions and all have their counterparts in animal pathology. These are problems of growth and in order to understand them it is
FIG. 3. (A) A primary and a secondary crown gall tumor on a sunflower plant (*Helianthus annuus* L.). (B) Tumors of the type that arise spontaneously in certain interspecific hybrids within the genus *Nicotiana* (*N. langsdorffii* × *N. suaveolens*). (C) Crown-gall teratomata on *Kalanchoe daigremontiana*. Note the tumor tissue has a tendency to organize. (D) Black’s virus-induced tumor on sweet clover (*Melilotus alba* Desr.). (Photographs by J. A. Carlile.)
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necessary to understand something of the processes involved in normal growth and development.

Growth in all animals and plants results either from cell enlargement or from the combined processes of cell division and cell enlargement. These two fundamental growth processes appear to be dependent for their development, in plant cells at least, upon specific substances that may be synthesized by, but are precisely regulated in, all normal plant cells. By varying the ratio of a factor limiting for cell enlargement and one limiting for cell division it is possible to obtain, with the use of certain normal cell types as a test object, either (1) a high degree of organization involving the production of numerous shoots, (2) a completely unorganized callus composed of hypertrophied and hyperplastic cells, or (3) the extensive formation of roots (Skoog and Miller, 1957). The effect of certain limiting factors on the growth of plant cells and on the development of plant organs is clearly evident from studies of this type. It is from this point of view that the three non-self-limiting neoplastic diseases of plants listed above will be discussed.

1. Crown Gall

The crown gall disease has been studied over the years more intensively than have other plant neoplasms. It has served as the experimental model in the field of plant oncology. Crown gall, which is initiated by a specific bacterium, affects plants belonging to at least 142 genera present in 61 widely separated botanical families. No monocot, however, shows unequivocal response to infection. The bacterium causing this disease possesses the ability to transform normal plant cells to tumor cells in short periods of time. Once the cellular alteration has been completed, the continued abnormal growth of the tumor cell becomes entirely independent of the causal bacteria. The cells of such a tumor continue to grow autonomously at the expense of the host and under favorable conditions the resulting growths may reach enormous size. Crown gall tumors weighing up to 100 lb. have been described in the literature. In certain hosts such as sunflower and Paris daisy there are produced, in addition to primary tumors, secondary tumors that develop at points distant from the seat of the primary inoculation. The secondary tumors are of interest because they are commonly free of the bacteria that initiate the primary growth (Braun, 1941; Smith et al., 1912). This finding permitted the unequivocal demonstration of the truly independent nature of the crown gall tumor cell (Braun and White, 1943; White and Braun, 1942). Tumor cells isolated from such sterile tumors and planted on a suitable culture medium grew profusely and indefinitely on that medium. While the sterile sunflower tumor tissues increased in theo-
The commonly found type of crown gall tumor is characterized both in the host and in culture (see Fig. 4, A) by the mostly undifferentiated and completely unorganized growth of its cells. In certain plant species, the cells of which possess highly developed regenerative capacities, there may however be produced, in place of the characteristic crown gall tumor, a complex overgrowth or teratoma which is composed in large part of highly abnormal leaves and buds that show varying degrees of morphological development. Sterile tissues isolated from such organized but abnormal structures grow profusely in culture, as do typical crown gall tumor cells, on a medium that does not support the continued growth of normal callus tissue, as shown in Fig. 4, B and C. Teratoma-derived cells differ from the commonly found crown gall tumor cells, however, in that they retain indefinitely, in culture, a highly developed capacity for organization. The surfaces of such cultures are covered with small, organized structures many of which appear morphologically to be leaves. Histologically, however, these structures are not usually made up of the well differentiated cell types of which leaves are normally composed. An attempt has been made to analyze more precisely the conditions which determine tumor morphology in crown gall (Braun, 1953). Of importance were (1) the strain of the bacteria used to transform normal cells to tumor cells, (2) the relative position that the altered cells occupy in a host, and (3) the inherent competence for regeneration possessed by the affected cells. It was also concluded from this study that the tumor-inducing principle elaborated by highly virulent strains of the bacteria completely overpowers the cellular factors concerned with differentiation and organization of pluripotent plant cells, while the principle associated with a moderately virulent strain is incapable of com-
pletely suppressing those factors. In the latter instance, the pluripotent teratoma cells retain, despite their conversion to tumor cells, highly developed regenerative capacities. The teratoma tissue is of particular interest because of its usefulness in studying the problem of recovery of crown gall tumor cells (Braun, 1951a).

![Images of crown gall tumor tissue](image)

**Fig. 4.** (A) Crown gall tumor tissue of the unorganized type planted on White's basic medium. (B) Crown gall teratoma tissue. This tissue retains indefinitely a capacity to organize highly abnormal leaves and shoots. (C) Normal tissue of the type from which the tumor tissues were derived. The normal tissue does not grow continuously on this culture medium. (Photographs by J. A. Carlile.)

It is now generally believed that all living nucleated cells of a plant—except perhaps those that are heavily lignified—can be transformed to crown gall tumor cells. Experimental studies have shown, however, that such cells must be conditioned or rendered susceptible to transformation as a result of the stimulus accompanying a wound. It is only during a limited period in the normal wound-healing cycle that normal
plant cells can be converted to tumor cells (Braun, 1952). Following wounding, the host cells gradually become susceptible, reaching a maximum vulnerability between the second and third days after a wound is made. Thereafter, the cells again become progressively more resistant as wound healing progresses toward completion. When a wound is made, the cells in the region of the wound are activated and some two to three days later they begin to divide to heal the wound. Under normal circumstances, upon repair of the wound, the cells which participated in the wound response return again to a quiescent state. In the case of crown gall this return to normalcy is blocked. Some as yet undefined morphogenetic restraint is no longer applied to, or if applied is no longer effective upon, the tumor cells of the regenerating tissues. As a result, the altered cells continue their proliferation in an unregulated and autonomous manner. This unregulated growth results in disorganization of the tissue, in hypertrophy and hyperplasia of the cells, as well as in cytological abnormalities frequently found associated with the tumorous state in crown gall. It is clear, therefore, that the cells proliferating in the tumor are no longer callus cells for they no longer behave as callus cells do. They represent new cell types that have acquired, as a result of bacterial action, new properties the most important of which is the capacity for continued unregulated growth in the absence of any recognizable infective agent.

Any attempt to account in physiological and biochemical terms for the abnormal behavior of a tumor cell must, if it is to receive serious consideration, also account for the morphological, histological, and cytological peculiarities that characterize the tumorous state. Such a concept has been developed and the evidence upon which it is based is outlined briefly below.

Since tumor cells generally appear somehow to have acquired a capacity for autonomous growth as a result of unregulated growth and division, the problem of autonomy has been explored by means of an analysis of the factors concerned specifically with growth accompanied by cell division. As indicated above, the fundamental growth processes of cell enlargement and cell division appear to be dependent for their development in plant cells upon specific substances that may be synthesized by such cells. Crown gall tumor tissue has been shown, moreover, to be a rich source of both a factor normally limiting for cell enlargement and one limiting for cell division. As a result of recent work (Jablonski and Skoog, 1954; Steward and Caplin, 1951), it is now possible to delimit these two growth processes under fully controlled experimental conditions, with the use of certain plant cell types as test objects. When, for example, tobacco pith parenchymal cells are treated with synthetic
growth substances of the auxin type such as naphthalene acetic acid, the pith cells enlarge greatly in size but they do not divide. It is only when a second growth factor such as 6-furfurylaminopurine—or the naturally occurring equivalent of that substance—is supplied to the pith parenchymal cells in addition to an auxin, that a profuse growth accompanied by cell division results. The 6-furfurylaminopurine without an auxin is ineffective in encouraging either an enlargement or a division of the pith cells. These findings demonstrate that two growth substances, one of which is concerned with cell enlargement and the other with cell division, act synergistically to promote growth and cell division in tobacco pith parenchymal cells. Normal tobacco pith cells do not and cannot themselves synthesize these two growth substances for, if they did, they would respond in the characteristic manner indicated above. Since the cellular systems responsible for the synthesis of these two growth substances appear to be solidly blocked in normal tobacco pith cells, a study was undertaken to learn how such cells would respond when transformed to crown gall tumor cells. The results of these studies, which are reported in detail elsewhere (Braun, 1956), demonstrated that when healing pith cells are converted to crown gall tumor cells, typical crown gall tumors develop. This simple experiment demonstrates that, although normal tobacco pith cells are not and cannot synthesize physiologically effective concentrations of either a cell enlargement or a cell division factor prior to their conversion to tumor cells, both substances are synthesized in greater than regulatory amounts following alteration. If this were not true, continued growth accompanied by cell division and, hence, tumor formation would not have resulted in the test system used in these experiments. It is clear, therefore, that an essential difference between a normal tobacco pith cell and a crown gall tumor cell appears to be concerned at a physiological level with the permanent activation of two growth-substances-synthesizing systems the products of which are specifically concerned with growth accompanied by cell division. The continued production in greater than regulatory amounts of the cell enlargement and cell division factors by the tumor cell could account for the continued abnormal proliferation of such a cell. Subsequent studies have shown (Braun, 1957a, b), however, that additional metabolic systems are permanently activated during the transition from a normal cell to a fully altered, rapidly growing type of crown gall tumor cell. It has been found that alteration of normal cells to tumor cells is a gradual but progressive process (Braun, 1943, 1951b). When, for example, the tumor-inducing principle responsible for inception of the crown gall tumor is allowed to act on plant cells for only 34–36 hours before being inactivated by thermal treatment, small, very slowly growing benign
growth are elicited in a host. A 50-hour exposure of cells to the action of that principle results in tumors that grow at a moderately fast rate. If the tumor-inducing principle is allowed to act for 72–96 hours before being destroyed by heat, rapidly growing (potentially malignant) tumors result. It is also possible to obtain tumors showing varying degrees of neoplastic change by allowing the tumor-inducing principle associated with slightly virulent or moderately virulent strains of the crown gall bacteria to act on host cells throughout a 4- or 5-day period. Sterile tissue isolated from the three types of tumors described above and planted on White’s basic culture medium retain indefinitely their char-

![Table showing different growth rates of tumor tissue](image)

Fig. 5. Relative rates of growth of 3 clones of crown gall tumor tissue that show different degrees of neoplastic change, planted on White’s basic medium. (Left) Fully altered, rapidly growing tumor cells. (Upper left) Moderately fast growing tumor cells. (Upper center) Slowly growing tumor cells. (Upper right) Normal cells of the type from which the tumor cells were derived. While the 3 clones of the tumor cells grow continuously, although at different rates on the basic culture medium, normal cells of this type do not grow on that medium. Lower pictures and legends show minimal nutritional supplements needed by the 3 types of tissues to achieve a growth rate comparable to that of the fully altered tumor cell. (Photographs by J. A. Carlile.)
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characteristic growth patterns. This is illustrated in Fig. 5. Since these three types of tumors are derived from the same plant species, they are admirably suited for a study of the factors required for rapid autonomous growth. In these studies, the results of which are summarized in Fig. 5, the rapidly growing fully altered tumor cell was used as the standard. This cell type can synthesize in optimal or near optimal amounts all of the growth factors needed for its continued rapid abnormal growth from mineral salts and sucrose present in White's basic culture medium. The moderately fast growing tumor cell required that the basic medium be supplemented with glutamine, meso-inositol, and a cell enlargement factor (naphthalene acetic acid) to achieve a growth rate comparable to that of the fully altered rapidly growing type of tumor cell. The very slowly growing benign tumor cells altered in a 34-hour period required, in addition to the three compounds described above, asparagine as well as cytidylic and guanylic acids to achieve full growth. It is clear from experiments of this type that, as the crown gall tumor cell becomes more autonomous, its requirements in terms of externally supplied growth factors become less exacting. These studies demonstrate clearly, moreover, that a series of well defined growth-substance-synthesizing systems become gradually activated during the transition from the normal cell to the fully altered tumor cell, and the degree of activation of these systems determines the rate of growth of the tumor cell.

Normal cells of the type from which the tumor cells are derived do not grow on the basic medium. Thus, although the difference between the three types of tumor cells is quantitative since all grow continuously, though at different rates, on the basic medium, the difference between the tumor cells and the normal cell is qualitative. One qualitative difference found to exist in these studies is the absolute requirement of the normal cell for 6-furfurylaminopurine or the naturally occurring equivalent of that substance. The addition of that compound to the basic media or to the supplemented culture media did not stimulate growth of any of the tumor tissues. The normal cells also possess, in contrast with the tumor cells, an absolute requirement as a supplement for a cell enlargement factor such as naphthalene acetic acid. The addition of 6-furfurylaminopurine and naphthalene acetic acid to the basic medium permits the very slow growth of normal cells. However, only if the basic medium is supplemented with glutamine, asparagine, inositol, guanylic and cytidylic acids, in addition to the auxin and 6-furfurylaminopurine, do the normal cells achieve a growth rate comparable to that of the fully altered, rapidly growing type of tumor cell. It thus appears that, as a result of the transition from a normal cell to a fully altered, rapidly
growing crown gall tumor cell, a series of quite distinct but well defined growth-substance-synthesizing systems become progressively activated. This leads to the production by the affected cell of greater than regulatory amounts of these growth-promoting substances. The continued production in greater than regulatory amounts of these substances by the tumor cell could and most probably does account for the continued unregulated proliferation of such a cell. Precisely how the tumor-inducing principle associated with this disease accomplishes the simultaneous unblocking of several apparently distinct and quite unrelated metabolic systems remains unanswered. These results are nevertheless understandable if it is assumed that some as yet uncharacterized master reaction within the cell is specifically but gradually unblocked by the tumor-inducing principle and which, once activated, not only accomplishes the unblocking of several other growth-substance-synthesizing systems but also determines the rate at which the entire series of metabolic events concerned with growth and cell division proceeds.

The concept of growth autonomy presented above finds additional support in other directions. It has been possible to reproduce, under precisely defined experimental conditions and with the use of certain normal cell types as a test object, not only the morphological growth patterns (Braun and Näf, 1954) (slow and rapid disorganized growths, teratoma-like structures) but also the histological (hypertrophy and hyperplasia leading to disorganization and loss of function) as well as the cytological (multinucleate giant cells, etc.) (Skoog, 1954) abnormalities that characterize the tumorous state in crown gall. This was accomplished by varying the proportions of the cell enlargement factor and the factor limiting for cell division in an otherwise suitable culture medium on which the normal cells were planted. These artificially stimulated normal cells, in contrast to crown gall tumor cells, are self-limiting growths and when the externally supplied stimuli are removed, their growth promptly stops. The fact that such stimulated normal cells commonly show histological and cytological characteristics of true tumor cells but are themselves self-limiting growths indicates that the observed cellular abnormalities are the result rather than the cause of the tumorous state.

Quite possibly, a cell acquires the capacity for autonomous growth as a result of the permanent activation of a series of growth-substance-synthesizing systems the products of which are concerned specifically with growth accompanied by cell division. These systems are precisely regulated in all normal plant cells.

Here, then, is a rare example of a disease in which an infective agent
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in some as yet uncharacterized manner regularly converts normal plant cells into new cell types in short periods of time. Once the cellular transformation has been accomplished, the pathogen no longer plays a role in the continued development of the disease. The altered host cells become pathogenic by virtue of the fact that those cells have acquired a capacity to synthesize greater than regulatory amounts of growth-promoting substances of a type that are limiting for growth and cell division in normal plant cells.

Although growth-promoting substances elaborated by the tumor cells themselves appear to be responsible for the continued unregulated growth of such cells, other sources of growth hormone may influence the morphological growth patterns as well as the rates of growth of the resulting tumors. This is particularly evident when dealing with crown gall tumor cells that grow slowly and possess relatively low grades of neoplastic change. For example, the tumor-inducing principle elaborated by certain attenuated strains of the crown gall bacteria initiates very slowly growing tumors on plants such as the tomato. The application of synthetic hormones of the auxin type to such normally slowly growing tumors results in the formation of large rapidly growing tumors (Braun and Laskaris, 1942). In this instance, the effect of the growth-promoting substance on the cell is only temporary and, although growth is enhanced, the increased growth rate does not become an intrinsic property of the cell itself. Not only the rate of growth but also the tendency of tumor tissue to organize may be hormonally influenced. When, for example, the tumor-inducing principle elaborated by a moderately virulent strain of the bacterial pathogen transforms pluripotent cells at the cut stem tip of a tobacco plant, complex tumors or teratomata arise. When, on the other hand, this same tumor-inducing principle alters similar cells in an internode of a plant containing a functional apical bud, and hence hormone-producing center, typical unorganized crown gall tumors develop. In this instance, the functional apical bud can be wholly replaced in suppressing the tendency of the tumor tissue to organize by synthetic growth hormones. The effect of the hormone in suppressing organization in the tumor tissues is only temporary, as evidenced by the fact that tissue isolated from either the organized teratomata or unorganized tumors, when planted on a culture medium, shows identical growth patterns. In both instances, the tissues are characterized by a capacity to organize highly abnormal shoots and buds. It can, therefore, be concluded that the greater the degree of the primary change in a tumor cell and, hence, the greater the growth-substance output by that cell, the less effective are externally supplied hormones in stimulating growth.
The growth of fully altered, rapidly growing crown gall tumor cells is, in fact, inhibited when such cells are treated with growth substances of the auxin type.

2. Wound Tumor

A second plant disease which fulfills all of the criteria of a true neoplastic disease is one caused by a typical virus. This malady is known as the wound tumor disease. The etiological entity responsible for this disease is insect-transmitted and is one of a group of viruses that multiply in the insect as well as in the plant host.

On a large variety of plants (Black, 1949, 1952, 1954), the symptoms produced in response to the virus involve morphogenetic disturbances. A survey of potential hosts indicated that at least 43 species belonging to 20 plant families produce symptoms typical of the disease, including irregular enlargements of the veins in leaves and tumors on the roots. Other symptoms observed included leaf curling and distortion, leafy outgrowths from the undersides of the veins, vein tumors, distortion of petioles, and shortening of internodes with a resulting dwarfing of the infected plant.

The variations in symptoms vary from barely detectable to those that are extremely pronounced. In Portulaca the tops of the infected plants bear no symptoms, but the roots contain many small tumors. In some plants such as sorrel and sweet clover the response to infection involves the production of large tumors which in the case of sweet clover appear on the stem. Investigations on the latter host demonstrated that the hereditary constitution of the plant affects the number, size, distribution, and morphology of the tumors (Black, 1951). In some strains the root tumors may be so large and numerous that they fuse together, while in others so inconspicuous that they may easily be overlooked. Similarly, in some clones, stem reaction involves the formation of many large tumors; in others they are rarely found. Moreover, hereditary influences on the stem and root may act independently. It has been found, for example, that one clone produces large tumors on stems and roots while another produces many large tumors on the roots but only occasional tumors on the stem.

That the genetic basis which causes a clone to be highly predisposed to tumor formation involves not merely a susceptibility to the virus but also an inherent tendency toward tumorous proliferation is indicated by the findings of Littau and Black (1952). These investigators found that the inbred B21 clone of sweet clover, which responds actively with tumor formation as a result of virus infection, produced five spontaneous tumors over a period of several years. That these new growths were not the
result of accidental virus infection was adequately demonstrated. The authors have compared their B21 line of sweet clover with strain C3Hb of mice which have lost the mammary carcinoma virus and yet show a strong inherent tendency toward the development of mammary carcinoma.

In this, as in other neoplastic diseases of plants, wounding plays an important role in the initiation of the disease. Tumors develop from accidental or artificially made wounds, from points of stress, as well as in those regions where lateral roots emerge. Black and Lee (1957) have also demonstrated that when infected plants are treated with an auxin, a pronounced increase in the number and size of stem tumors results. Thus, at least three factors appear to be essential for the initiation and development of tumors in this disease: (1) the inciting virus, (2) a wound or some similar stimulus, and (3) the hereditary constitution of the host.

The function of the wound is not yet clear. Brakke et al. (1954) have suggested that perhaps the response of a cell to a wound permits an increase in virus concentration and that the high concentration maintains the cells in a state of active proliferation. The cells present in tumors of sweet clover have been found to contain about one hundred times more virus than do nontumorous portions of a diseased plant. Another possibility presents itself, however. It may be that virus-infected and hence potential tumor cells do not develop into a neoplastic growth until they are first stimulated to divide by noncarcinogenic processes such as wounding, application of hormones, etc. Once cell division is initiated as a result of a nonspecific stimulus, the infected cells are no longer subject to the morphogenetic restraints in a host which normally return the cell to quiescence after wound healing has gone to completion. Kelly and Black (1949) have raised the very pertinent question as to why one cell in the pericycle of an infected sweet clover root develops into an organized lateral root, while other pericycle cells in close proximity develop into disorganized tumors. There are several possible answers to this question. Since lateral root formation occurs earlier than tumor formation, it may be that the more embryonic tissue has such tight restraints placed upon it that the infection does not lead to its disorganization. On the other hand, lateral roots, initially at least, appear to involve the xylary pericycle while the tumors generally involve the phloic pericycle. These differences may reflect divergencies in morphogenetic response at the two sites. A third possibility rests on the assumption that only certain cell types are infected by the virus. The leaf hopper-transmitted viruses, of which the wound tumor virus is a representative, appear to show a predilection for phloem tissue. Since lateral root forma-
tion initially involves the xylary pericycle, it may be that at the stage of root development that permits lateral root initiation, only that portion of the pericycle closest to the phloem is infected with virus. It would, therefore, not be until lateral root formation involved tissue close to the phloem that a tumor would develop, because not until then would the cell division stimulus associated with lateral root formation involve virus-infected cells.

Once tumors are initiated, the virus remains closely associated with the tumorous tissue. These tumors possess a capacity for indefinite growth, both in the host and in culture. Sterile tumor tissue isolated from sorrel and planted on White's basic culture medium initially grew rather slowly and doubled its volume every 3 weeks. Subsequently, Burkholder and Nickell (1949) devised a more suitable culture medium which permitted far more rapid growth of the tumor tissue. The tumor tissue in culture was found to possess an unusually high requirement for phosphorus. This was interpreted to reflect the need of the multiplying virus for this substance. Similarly, an RNA hydrolyzate, and more specifically uracil, was found to exert a stimulatory effect on the growth of the tumor tissues.

There is no question about the fact that specific viruses are etiologically implicated in the wound tumor disease of plants as well as in certain virus-induced tumors of animals. The question that arises, however, is: How does the virus induce the neoplastic state in a host cell? Viruses cause cells of plants and animals to respond to infection in many different ways. Few elicit tumorous growths. Even the tumor-inducing viruses may infect cells and not exert a stimulatory influence on such cells. What is it then that causes certain viruses to induce cells to proliferate in an essentially unregulated and uncontrolled manner? There is a partial answer at least in the case of Black's wound tumor disease. The virus-infected tissue, like crown gall tumor tissue, acquires a capacity to synthesize growth-promoting substances concerned specifically with growth and cell division. This is evidenced by the fact that the tumor cells grow rapidly and indefinitely on a culture medium that is lacking in certain growth factors needed for the continued growth of normal cells. It thus appears that the presence of the virus confers upon the cell the ability to produce such growth-promoting substances in greater than regulatory amounts and as a result the cell achieves a physiological autonomy.

3. Genetic Tumors

The formation of non-self-limiting tumors, in the development of which no demonstrable infectious agent is involved, is a regular occur-
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rence in certain plants. In these instances, it is the genetic constitution of the cells within a plant that appears to be of importance. The most thoroughly investigated examples of this type involve the development of spontaneously occurring tumors in a large number of *Nicotiana* hybrids. Kostoff (1930) demonstrated that when appropriate *Nicotiana* species are crossed, tumors develop in all hybrid offspring. These hybrid plants are interesting because they are, for the most part, perfectly organized both morphologically and histologically during their period of active growth and in the absence of irritation. Once such plants reach maturity and terminal growth ceases, a profusion of tumors may develop on all parts of the plants. It is, however, possible, according to Kunkel (1954), to initiate a tumor at almost any period in the development of a plant by making a wound in the vicinity of a vascular bundle. The cells of such plants behave as do normal cells until they are stimulated to divide. Once the cells of this plant are activated as a result either of natural processes or artificially induced irritation, they no longer respond to the morphogenetic restraints that return a normal cell to quiescence. The genetic constitution of the cell, in this instance, is critical. Only such a nonspecific stimulus, such as irritation, is required to transform the potential tumor cells, of which the hybrid plant is composed, into actively proliferating autonomous plant-cell types.

Since Kostoff's original description of this phenomenon, the number of tumor-producing combinations has increased considerably (Kehr, 1951; Kehr and Smith, 1954; Naf, 1958). The tumors apparently arise in tissue that has been stimulated to division by natural or artificial processes and, once initiated, the tumors retain the capacity for unlimited disorganized growth both *in vitro* and *in situ*. Normally, tumors on the stem develop at the site of leaf or petal abscission. A number of hybrid combinations have been found to produce tumors on the roots only. In those many instances in which tumors develop on all parts of the plant, tumor formation on the root often precedes the formation of overgrowths on the shoot. This increased response on the part of the root may result for several reasons. Lateral root formation may represent a stimulus similar to wounding and may, therefore, play a role in tumor initiation similar to that found in the virus tumors. Irradiation of hybrid plants hastens the onset of tumor formation and increases significantly the number of tumors that develop (Sparrow *et al.*, 1956). Chemical irritation may also initiate tumors. Kehr and Smith (1954) reported that leaves of certain hybrid combinations accidentally sprayed with a mixture of turpentine, whiting, and white lead, produced tumors at almost every spot where droplets of the spray mixture fell on a leaf.

The genetic tumors appear to represent a lower grade of neoplastic
change than do most crown gall tumors. This is evidenced both by the fact that the stem tumors show a strong tendency to organize abnormal structures and that they seldom, if ever, reach the size and state of disorganization of crown gall tumors initiated by highly virulent bacteria. Although these tumors are almost never directly fatal to the plant, they do represent a considerable burden to the plant.

Kehr and Smith (1952, 1954) attempted to analyze the precise genetic basis of these tumors by breeding a considerable number of diploid and polyploid combinations of *Nicotiana glauca* × *N. langsdorffii*. From the data obtained it was concluded that the tumor-forming nature of the hybrid remains relatively unchanged regardless of the ratio of *N. glauca* and *N. langsdorffii* genomes as long as at least one complete genome of each species is present in the hybrid. It was found, further, that, although spontaneous genetic tumors develop when all twelve *N. glauca* chromosomes are combined with a diploid complement of *N. langsdorffii* chromosomes, no tumors developed in hybrid plants when only one or a few *N. glauca* chromosomes are present in addition to the diploid *N. langsdorffii* genome.

Recently Näf (1958) has approached the problem from a different point of view. This worker has divided all of the parents of tumorous hybrids into two groups which he arbitrarily designated as “plus” and “minus.” Näf found that if an intragroup cross is made either between two “plus” species or two “minus” species, the offspring never develops tumors. On the other hand, crosses made between a “plus” species and a “minus” species produce tumorous offspring. Of a total of more than 50 crosses tested, very few exceptions to the above rule were found. Näf explains these exceptions on the basis of relative “plusness” and “minusness,” similar to Hartmann’s concept of relative sexuality. It was concluded from these studies that the critical contributions to tumor formation of the “minus” parents differ from those of the “plus” parents. These contributions, since they are of a genetical nature, must be reflected in parental metabolism and it should be possible to characterize them on a physiological level.

As in the case of crown gall and Black’s virus tumor, tissue isolated from the hybrid tumors and planted in culture is capable of synthesizing all of the growth factors required for its continued abnormal growth from mineral salts and sucrose present in a basic culture medium. What is particularly interesting in the case of the hybrid is that cells obtained from nontumorous portions of hybrid stems also become autonomous upon isolation and culture. Such tissue is truly tumorous and, as White (1944) has shown, it can be grafted to one of the parents, *N. glauca*, where the implants again develop into tumorous overgrowths. Hybrid
tumor tissue does not commonly differentiate and organize when grown on a semisolid medium. White (1939) found, however, that when such tissue is immersed in a liquid medium, it tends to organize shoots and leaves. It was presumed that oxygen gradients influenced the differentiation process. Skoog (1944) found, however, that the tendency to form buds and leaves can be completely suppressed by the addition of 0.2 p.p.m. of indoleacetic acid. Low concentrations of indoleacetic acid were found not only to suppress organization but also to stimulate growth. The indoleacetic acid effect was reversible by raising the level of certain nutrients such as sucrose, KH₂PO₄, and Fe₂(SO₄)₃. Subsequent studies on tobacco (Skoog and Tsui, 1951; Miller and Skoog, 1953) demonstrated that bud formation on isolated stem segments of tobacco depends upon an adenine:indoleacetic acid ratio. Adenine favors bud development which, in turn, may be inhibited by the addition of indoleacetic acid. More recently, Skoog and Miller (1957) have reported that 6-furfurylaminopurine is far more effective than adenine, in this respect.

The capacity of hybrid tumor tissue to organize shoots and buds under certain conditions does not reflect a tendency of such tissue to return to normalcy since these organized structures are composed entirely of potential tumor cells and they would appear, therefore, to be organized tumors.

The following picture emerges from studies on the non-self-limiting neoplastic diseases of plants. It is apparent from this discussion that several quite distinct agencies can bring about the tumorous state. The biological aspects of this state are characterized by growth autonomy. This phenomenon can be adequately explained on the basis of the ability of the tumor cell to synthesize certain growth-promoting substances in greater than regulatory amounts. The rate of growth of a tumor cell appears, moreover, to be a function of the degree to which the growth-substance-synthesizing systems within a cell are activated. The ultimate mechanism by which the growth-substance-synthesizing systems become activated in plant tumor cells is unknown. The production of these substances by the cell is, however, presumably enzymatic in nature.

Since enzymatic reactions are commonly considered to be gene-controlled processes, it might appear that the normal gene complement is somehow modified in the plant tumor cell. This could conceivably be accomplished by somatic mutation at the genic level. There are several reasons for questioning somatic mutation as being the cause of the physiological autonomy that underlies the tumorous state in plants. The first of these is that a specific virus has been implicated etiologically in one of the diseases in question. It might be argued, however, that the virus induces a mutation at the genic level in cells and that once this
change is effected, the virus is no longer needed for the continued abnormal growth of the tumor cell. While this possibility cannot at present be ruled out in the case of Black's virus tumor, other closely related viruses that produce abnormal growth patterns in plants have been eliminated from their hosts by thermal treatment with a resulting complete recovery of such plants (Kunkel, 1936, 1941). Plant viruses of this type do not appear, therefore, to induce permanent modifications in plant cells. The continued abnormal behavior of the cell seems to be dependent upon the continued presence of the virus. In this respect, the virus-induced tumors behave as does the clubroot disease of the crucifers in which continued cell stimulation is dependent upon the continued presence of the pathogen.

The reported recovery of crown gall tumor cells also appears to argue against somatic mutation as the cause of the physiological autonomy found in this type of tumor cell. By forcing abnormal tumor buds present in crown gall teratomata into very rapid growth by a series of graftings to healthy plants, a gradual but ultimately complete recovery of such cells was achieved. These findings suggest that crown gall tumor cells may recover if they are forced to divide with unusual rapidity at the stem apex. They suggest further that the factor responsible for the continued abnormal proliferation of the crown gall tumor cell is an autonomous or partially autonomous entity that is subject to the effects of dilution in cells that are forced to divide with great rapidity (Braun, 1954).

The findings reported above are very suggestive of some encountered in microbial genetics. Studies such as those presented by Ephrussi (1951), Sonneborn (1946), and Spiegelman (1954) indicate that certain self-duplicating cytoplasmic factors as well as the nuclear genes may serve as determinants of hereditary differences in a cell. Certain of these cytoplasmic entities appear, moreover, to be concerned with enzyme production. Therefore, mechanisms quite different from somatic mutation at the gene level can be postulated to explain the continuity of tumorous properties from one cell generation to the next. It may well be that it is in the particulate cytoplasmic fraction of the cell rather than in the nucleus that changes occur which account for the physiological autonomy that underlies the tumorous state in plants.

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