I. Harmonious Changes Involving Exaggerated Growth Responses

A. GENERALIZED STIMULATION

One of the most characteristic features of higher plants and animals is the extraordinary way in which all their functional parts fall into a coherent, flexible but definitely limited pattern. In the normal organism, 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 an organism certain proportions that remain constant from one generation to the next. Sometimes, however, this harmony of structure and function is brought to our attention by its sudden failure. Such divergence from the normal may represent only slight quantitative changes which are evidenced by harmonious but somewhat exaggerated manifestations of normal developmental processes. This sort of response is well illustrated in the bakanae disease of rice (Oryza sativa), which is caused by Gibberella fujikuroi, as well as in several other plant diseases that are caused by the rusts (15). In those instances the host metabolism is affected by virtue of the fact that excessive amounts of the gibberellins are synthesized. Certain other plant diseases give indication that the process of infection interferes either with the synthesis or with the utilization of the gibberellins. Carrot (Daucus carota var. sativus) plants infected with the tomato big bud virus of California were shown by Kunkel (41) to bolt and flower prematurely. These findings suggest that virus infection may increase the synthesis of the gibberellins by the
hos: species. On the other hand, the rosette diseases, as exemplified by peach and peanut rosette viral infection, may interfere with the synthesis or utilization of such growth regulating substances by the plant. It has, in fact, been possible to reverse the stunting effects, but not other symptoms, associated with aster yellows and the corn stunt disease by applying gibberellic acid to an infected plant (60).

Nastic responses, which refer to changes in position of a bilaterally symmetrical organ as a result of differential growth, are a common manifestation of certain diseases. Dimond and Waggoner (26) made a detailed study of the cause of epinasty in *Fusarium* wilt of tomatoes (*Lycopersicon esculentum*). It was concluded from those studies that ethylene is responsible for the characteristic growth response in that instance. This conclusion was based on the observation that ethylene is produced in culture by the causal fungus. Ethylene production by infected tomato plants was, moreover, demonstrated by confining such plants with healthy indicator plants and by showing that nastic responses developed to a greater degree in the indicator plants than when such plants were confined with healthy tomato plants.

Another characteristic response involving growth movements is concerned with the upright growth habit of leaves. This is one of the most characteristic symptoms of the aster yellows disease. In plants infected with that virus the petioles elongate and assume an extremely upright growth habit which approximately parallels the main axis of the plant. It is this upright growth habit that suggested the trivial name “rabbit ears” for lettuce (*Lactuca sativa*) plants infected with that virus. Hypoanasty has also been observed in plants affected with the bacterium *Erwinia phytophthora*.

The development of organs that arise in unusual places has been reported to be a characteristic response of plants infected with bacterial, fungal, and viral diseases.

Carrot yellows virus from Texas as well as the aster yellows virus stimulate the production of small aerial tubers in the axils of the leaves of potato (*Solanum tuberosum*) plants. The witches’ broom virus, on the other hand, causes the infected plant to produce numerous buds at the nodes in the aboveground stems of potato plants. Long slender stolons that resemble aerial roots, but which are covered with trichomes, develop from these adventitious buds.

The development of adventitious roots on stems of plants has been reported to be a characteristic response in tomato following infection with *Pseudomonas solanacearum*, *Corynebacterium michiganense*, *Fusarium oxysporum*, *F. oxysporum f. lycopersici* (*F. lycopersici*) as well as the cranberry false-blossom virus. An extreme example of this is
found in the hairy-root disease of apples (*Malus sylvestris*) (see Fig. 1).

Grieve (30) suggested, as a result of his studies on the Granville wilt disease of tomatoes, that adventitious roots very probably result from disturbances of normal auxin transport in the host as a result of mechanical blockage rather than from indoleacetic acid elaborated by the bacteria. In the case of *Fusarium* wilt of tomato, Dimond and Waggoner (26) indicate that adventitious root formation, like epinasty, may result from the production of ethylene either by the causal fungus or by the cells of the tomato plant as a result of the interaction of the host and pathogen.

### I. Modification of Leaves

The curling and distortion of leaves is so characteristic of certain plant diseases that such maladies as leaf roll of potatoes, curly top of sugar beets (*Beta vulgaris*), and peach leaf curl have been assigned their trivial names because leaf curling is the most conspicuous feature of those diseases. Peach leaf curl, which is caused by *Taphrina deformans*, has been studied intensively. In that instance the causal fungus infects very young leaves which soon become curled and puckered, have a firm
consistency, and increase greatly in thickness. The host cells which are in the vicinity of the fungus are stimulated to enlarge and, to a lesser extent, to divide. The cells of the palisade parenchyma respond much more actively to the stimulus of the fungus than do the adjacent cells of the spongy parenchyma, and this leads to differential growth with a resulting puckering and curling of the leaf. Such morphological abnormalities are doubtless due to hormonal imbalances resulting from the infection by the fungus.

2. Frenching

A deformity of tobacco (Nicotiana tabacum) which is commonly known as frenching is found in most tobacco growing regions of the world. An advanced stage of this condition is characterized by a cessation of terminal bud and stem growth. When apical dominance is lost, the buds in the axils of the leaves develop and unusually large numbers of leaves, which may number as many as 300, appear on a plant. Such leaves are characteristically sword- or string-shaped because of failure of the leaf blades to develop. Although the cause of frenching has not been unequivocally established, it is now believed to be a toxicity disease rather than to result from nutritional deficiency. No parasitic organism of any kind has as yet been implicated in this condition.

Steinberg (92, 93) has suggested that frenching of tobacco is caused by a toxic metabolite elaborated by the widely distributed nonpathogenic soil bacterium Bacillus cereus. The progressive development and type of symptoms produced under controlled conditions in tobacco plants by the toxic metabolite largely paralleled those found in frenched plants in the field. In later studies Steinberg (94) found that the natural isomers of isoleucine and alloisoleucine, when used in concentrations of 2–8 ppm, produced a strapping of leaves of young tobacco plants. This worker found, moreover, that frenching was accompanied by a significant increase in isoleucine as well as certain other free amino acids in the leaf lamina of field grown plants (95). The absence of free amino acids in soils that leads to frenching and the marked increase of free isoleucine in frenched leaves indicate that the Bacillus cereus toxin and isoleucine are not identical. It was therefore concluded that the accumulation of excessive amounts of free amino acids in strapped leaves was a probable chemical factor involved in the production of the morphological abnormalities observed in frenched tobacco plants.

3. Alterations in Floral Parts

Under the influence of pathogenic agents of various types the sepals, petals, stamens, or pistils of a flower may be altered into structures that
are very different in appearance from those found normally. In the United States there are at least three different viruses—the aster yellows virus, the tomato big bud virus, and the cranberry false blossom virus—that produce gigantism in the sepals and calyces of certain solanaceous plant species. The tomato big bud virus appears to affect the sepals of tomato flowers rather specifically (see Fig. 2). These structures enlarge greatly under the influence of the virus and fuse to form huge bladderlike structures that may be ten times or more the normal size.

Viruses of this type do not cause gigantism in the sepals of certain other plant species. In Catharanthus roseus (Vinca rosea) such viruses bring about a green coloring of the petals, stamens, and styles. There is, in this instance, a retrograde development of floral parts into foliage leaves.

Metamorphoses of floral parts of higher plants are also accomplished by certain species of fungi. The transformation of stamens into petals commonly follows infection by the rust Puccinia violae on Viola sylvestris. Stamen primordia found in flowers of Knautia arvensis develop into petallike structures following infection by Peronospora violacea. Many other such examples could be cited.
Bos (7) has interpreted the development of the flower and its component parts on the basis of the phenomenon of antholysis accompanying virus infection in *Crotalaria*. This investigator has suggested on the basis of the homology of reproductive and vegetative parts of a plant and their morphogenetic development that the vegetative and sexual activities in a plant are mutually antagonistic. A growing point can develop only into a vegetative shoot or into an inflorescence. During flower initiation, the vegetative characteristics are suppressed and the sexual characters prevail. The reverse is also true. From the manner of appearance of antholysis in virus-infected *Crotalaria* plants, Bos has concluded that flower induction is suddenly stopped and the subsequent development of floral parts proceeds exclusively in a vegetative manner. The ultimate result depends on the stage of the development of the primordia in the bud at the time of the suppression of flower induction by the virus. Thus, flowers initiated in succession produce a series of flowers showing increasing antholysis. These in reverse order show a macroscopically recognizable picture of the morphogenesis of the flower and its component parts. Therefore, according to Bos, antholysis supports the theory that the flower must be regarded as a modified leafy branch.

In addition to modifying floral parts, infection with certain fungi releases growth restraints and thus overcomes the normally arrested development of such parts. For example, the anther smut, *Ustilago violacea*, produces its spores only in the anthers of certain plant species. When that fungus establishes itself in the female flower of *Melandrium album* or *M. dioicum*, the stamens, which are normally arrested in their development and are found only in rudimentary form in the pistillate flowers, grow to full size and when mature are filled with smut spores rather than with pollen. The nature of the morphogenetic stimulus that overcomes the arrested development of the male sex organs in female flowers remains unknown.

II. Amorphous Changes in Growth Pattern

A. Self-Limiting Overgrowths

1. Galls

Localized overgrowths in which the host cells are stimulated to excessive growth by a variety of disease-producing agents are known as galls. There are unusually large numbers of distinct overgrowths of this type to be found in plants. Among the more representative types are the insect and nematode galls, the cedar apples produced by the apple
rust on the juniper (*Juniperus*), *Pteridermium* galls on pine (*Pinus*), the so-called smut "balls," the black knots on plum and cherry (*Prunus* spp.), the overgrowths due to certain species of slime molds and chytrids, the galls produced by *Pseudomonas savastanoi* on the olive (*Olea europaea*), and the nodules that result in certain species of leguminous plants from infection by bacteria of the genus *Rhizobium*. It is, of course, not possible to consider in detail in this chapter all the overgrowths of this type, and 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 (44), Ross and Hedicke (79), and more recently by Felt (29) suggests that many of these highly specialized overgrowths represent beautiful examples of dependent differentiation.

Insect galls may result from either a mechanical or a chemical stimulus. Chemical stimulation 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 host attacked by different but closely related insect species produces morphologically very different galls. Figure 3 illustrates this point and shows drawings of three galls of quite different morphology induced on leaves of a western species of oak by three closely related species of cynipids. The morphology of the

Fig. 3. Three morphologically distinct galls produced on leaves of the California white oak by three closely related species of insects. (Drawings by R. J. Mandlebaum.)
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 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 (*Salix*). 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.

It is, of course, not possible to describe in detail in a chapter such as this all the diverse morphological structures that have been described for insect galls. Only a few interesting types within each of the larger groupings will be considered. Insect galls have been classified as: (i) simple galls in which a single animal or colony of animals is limited to a single plant organ, and (ii) compound galls where two or more plant organs may be concerned in the production of the gall. For convenience of description, simple galls have been divided into: (a) felt galls, (b) mantle galls, and (c) solid galls. The felt galls result chiefly from hypertrophied epidermal cells growing out into hairy coverings of various sorts and shapes. Many felt galls are caused by gall mites. They form cottony growths on limited and sharply defined areas, usually on the undersurface of the leaf. Microscopic studies have shown that in the formation of the felted galls the epidermal cells swell out and become transformed into bent and twisted tubes that may be shaped like a club. The mites which produce the felt galls deposit their eggs in elongated cells and their young live on the materials contained within the cells.

The mantle gall is a hollow structure which always has a portion of the surface of the affected organ for its lining. This type of gall, which may arise in various ways and assume a variety of forms, is in effect a chamber formed by hypertrophied growth around the place occupied by the insect. Thus, the insects that give rise to galls of this type live on the surface of the leaves where they multiply and attach their eggs to the epidermis. The growth stimulus produced by the insect excites the leaf tissue to growth which forms a protecting mantle for the animal. The mantle galls have been divided according to structure into scroll
galls, pocket galls, and covering galls. Scroll galls usually involve the blades and more rarely the petioles of the leaves. As a result of the presence of these insects on a leaf surface the leaf grows more profusely on one side than on the other. This causes the leaf to curl lengthwise and form a scroll or chamber in which the animals are embedded. It is always the side of the leaf on which the insects live that becomes concave. In many instances the whole leaf lamina is rolled. In other cases the alteration is restricted to the margins of the leaf.

The pocket galls are closely allied to the scroll forms. In galls of this type the tissue of the leaf lamina or petiole is subjected to the stimulus from the animals with the result that a hollow protuberance arises, the cavity of which serves to protect the insects. Pocket galls exhibit a great variety of form and shape, some being wrinkled or plaited; others, saclike structures attached to the leaf by a very narrow neck. Many of these galls project from both the upper and lower side of the leaf. The pocket gall of the sloe (*Prunus spinosa*), caused by a gall mite, projects almost as much from the underside as from the upper side of the leaf. On the other hand, a similar gall on the bird cherry (*Prunus padus*) rises on the upper side as a long pocket but projects on the lower side only as a small wart.

The third type of mantle gall is the covering gall. In this instance the tissue begins to grow at the point at which the insect has settled or where an egg has been fastened to the epidermis. The tissue rises up in the form of a fleshy wall which continues to grow until the animal is roofed in. The cavity in this instance arises from an overarching of the tissue.

The third group of the simple galls is the so-called solid galls. These are produced by insects which pierce the plant tissue and lay their eggs in the wound. The common gall with the mosslike covering occurring on the wild rose (*Rosa*) and caused by the gall wasp *Rhodites rosae* is an example of this type. The wasp deposits its pointed or sometimes hooked eggs early in the spring into an undeveloped leaf which is still folded in the bud. The first sign of alteration in leaf structure following insertion of the eggs is the development of numerous hairlike processes. When the eggs hatch, the larvae penetrate deeper into the tissues of a leaf and the stimulated leaf cells grow out into a solid gall containing as many chambers as there are larvae. Hairs continue to form on the exterior until the characteristic structure of the gall results.

The structure of the wall of the larval chamber in the solid galls may differ in the galls induced by different insects. There is, however, always a layer of thin-walled cells immediately surrounding the egg, which is known as the medulla or pith, and an outer layer composed of cortex
and epidermis which surrounds the inner layer like a skin or bark. In many instances there is a third layer inserted between the pith and cortical layer which is composed of very hard cells and which forms a protective layer. The medulla or pith is composed of thin-walled cells rich in nutrients. These cells provide the necessary food for the larvae. The cells of the gall pith remain capable of division as long as the larvae require food and the cells are replaced almost as soon as they are eaten.

In the solid galls the hard, cortical, and epidermal cell layers are modified in many ways to protect the galls from drying out as well as to protect them from birds and other animal predators. Most solid galls completely enclose the chamber in which the larva dwells. The insect when it emerges must itself make an exit passage from the gall. The gall wasp and certain other insects do this by biting a hole through the gall with their mandibles. In other instances far more subtle methods of escape have been developed. The openings in some galls, which resemble operculate capsules, are especially remarkable. The gall gnat Cecidomyia cerris produces a solid gall on the turkey oak (Quercus cerris). This gall is so embedded in a leaf that it projects on the upper side as a small pointed cone and on the lower side as a disk covered with a thick coating of hairs. In the autumn a circular piece like a lid which is sharply defined becomes detached from the lower side of the chamber. The operculum falls out of the gall and the larva drops to the ground, where it pupates. Another remarkable example of this type is found in galls produced on the green cortical tissue of young twigs of Schinus longifolia by Cecidoses eremita. This gall is spherical and quite hard. When the time approaches for the larva to leave the gall, a plug with a projecting rim is developed on the side of the gall farthest from its point of attachment to the plant. When the plug drops out, a circular hole is left which leads into the gall chamber, as shown in Fig. 4. It is through this hole that the larva escapes from the gall.

Very little is as yet known concerning the precise mechanism by which the solid galls are produced. Nevertheless, Lewis and Walton (49) reported some very interesting histological and cytological findings dealing with a biologically active substance believed to be involved in the growth and differentiation of cells of a solid gall of witch-hazel (Hamamelis virginiana) known as the cone gall. This gall is produced by the aphid species Hormaphis hamamelidis 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
natur 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
are embedded minute crystalloids. These crystalloids show a reddish-
purple cast in such stains as gentian violet, congo red, and acid fuchsins.
They are Feulgen positive. They are, moreover, capable of passing
readily from cell to cell as well as of entering cells when they are
deposited by the insect in the intercellular 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 crystallloid
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
apparently incapable of self-propagation and since they appear to be
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.
Continued development of the gall is, therefore, dependent upon con-
tinued presence of the Feulgen-positive material.

Boysen-Jensen (8) studied the development of a midge (Mikiola
fagi) gall on beech (Fagus) leaves. The evidence presented supports the idea that the formation of the gall in this instance is caused by the 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 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 highly differentiated and organized cynipid galls.

The compound insect galls originate when several organs of a plant immediately adjacent to one another are concerned in the production of the overgrowth. Such compound galls commonly arise from either vegetative or floral buds. They are so varied in their character that it is difficult to classify them.

The ordinary vegetative bud galls may be of essentially two types. The first is leafless or more correctly a gall in which the leaves are transformed into knob- or peglike structures which merge into the swollen axis that contains the larva. The second type possesses leaves, the gall being covered by more or less fully developed green foliage leaves or with scalelike bracts.

Certain species of gall gnats lay their eggs in flower buds. The larva hatched from an egg lives in the cavity of the ovary which becomes the larval chamber. The corolla which covers the ovary in the flower bud remains closed and serves as a cap for the larval chamber. The calyx may become inflated, enlarged, and fleshy. The gall in this instance resembles a small bulb.

Closely allied to the bud galls are the galls known in Central Europe as “cuckoo-buds.” These growths are characterized essentially by the fact that they involve only the base of the shoot, while the upper portion of the shoot continues its growth unaltered. The best known and most widely distributed gall of this type is produced by the aphid Chermes abietis on the twigs of the spruce (Picea) tree. In the early spring the parthenogenetic females attach themselves and lay eggs at the base of a young leaf. The larvae that develop from the eggs penetrate
the shoot with their beaks. The shoot swells considerably, as do the bases of the needles and the spruce gall results. The gall contains a series of cavities or chambers in which the larvae live. Usually in late summer the gall dries up, each small cavity opens by a slit, and the winged adults leave the gall. This gall resembles a small fir cone. That a morphogenetic stimulus is elaborated by the spruce-fir aphid is suggested by the fact that the aphid attaches itself firmly by its beak to a scale of a fir (Abies) bud and thus can directly influence only a few cells of the young shoot. Nevertheless, thousands of cells of such a shoot are affected and soon begin to assume an altered form and are stimulated to growth, indicating that a diffusible stimulus is active.

Cluster galls are bud galls in which the axis is greatly restricted and densely covered with leaf structures. It is between the crowded leaves of these galls that the insects involved pass part or all of their life cycle. Morphologically very different types of galls are produced, depending on whether the free ends of the leaves turn back or remain in contact with each other and whether the axis from which the leaves are derived is more or less restricted. Sometimes galls of this type appear as open rosettes, while at other times the leaves all fold together in a ball like the leaves of a head of cabbage.

Certain of the insect galls are said to bear, superficially at least, remarkable similarities to fruits, berries, nuts, and cones of various plants. The currant gall produced on the male catkins of the oak (Quercus) by Spathegaster baccarum bears a striking resemblance to the red currant berry not only in form and size but also in its red color. The galls produced on the foliage of the beech by the gall gnat Hormomyia fagi resemble small plums. In this instance there is a hard stony kernel containing the larva and a succulent outer layer that can be compared to the fleshy part of the plum. If the leaf rudiments of the Pistacia shrub are affected by plant lice of the species Pemphigus cornicularius, the rudiment assumes the form of a carpel and becomes fashioned into a hollow body which has the appearance of a pod. This pod is not at all like the fruit of the Pistacia shrub but bears a considerable resemblance to the pods found on an entirely different plant species, Ceratonia siliqua. Similarly, the type of metamorphoses resulting from the action of the gall gnat Lasioptera juniperina on the topmost leaves of the juniper assumes a form very much like the fruit of the arborvitae (Thuja).

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 (6) has suggested that an insect gall is almost comparable in its “determinate” growth to a leaf or a fruit. These galls
are of constant 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 usually not below that of the host. A fascinating field lies open here for exploration at the morphogenetic and biochemical levels.

b. Root nodules. In addition to the insect galls there are a number of other self-limiting overgrowths that are highly organized and specialized structures. The root nodules found to arise on many species of leguminous plants as a result of infection by bacteria of the genus *Rhizobium* represent an example of this type. The typical root nodule is composed of four histologically well defined zones. 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 commonly free of bacteria. The provascular tissue of the nodule arises as a result of the radial divisions and differentiation of cells at the periphery of the inner infected cells and the nodular cortex at the time when the nodule is still meristematic. These differentiate later into typical vascular bundles which consist 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 parenchyma cells, infected and noninfected, and is known as the bacteroid zone. It is in this so-called bacteroid zone that nitrogen fixation occurs. This region of the nodule also contains four very interesting pigments one of which, leghemoglobin, is closely related chemically to blood hemoglobin. Attempts have been made throughout the years to explain in physiological terms the mechanism involved in the development of the nodule. While it has been possible to produce overgrowths in certain leguminous plants by applying growth substances of the auxin type, it is, nevertheless, true that histologically these artificially induced growths do not bear the slightest resemblance to nodules produced under the stimulus of the bacteria. Thimann (104, 105) has shown, however, that auxin production in the nodule roughly parallels the growth of the nodule. Allen and Allen (2) pointed 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 increased growth substance content of the nodule, a suitable explanation concerning the role that auxin and
other growth-regulating substances play in directing the organization of the nodule is not yet at hand.

c. Root knot. Another self-limiting overgrowth that shows a degree of cellular specialization is caused by nematodes of the genus *Meloidogyne* and is known as root knot. The overgrowth produced on roots of many cultivated and wild plant species may appear as scattered tubercles or as extensive swellings which may reach diameters up to 2 inches and involve almost the entire root system.

Numerous studies devoted to the cytology and fine structure have provided an account of the sequence of changes associated with the initiation and development of the knot. Such studies have demonstrated that the larval form of the parasite penetrates the root near the growing point and moves both between and through the cells until becoming sedentary during the latter part of the second developmental stage of the parasite. As feeding begins, the walls of certain host cells dissolve and the contents of such cells coalesce to form multinucleate giant cells or syncytia. Commonly, 6 to 20 nuclei are present in the syncytia although Nèmec (68) reported more than 500 in a single cell of *Cissus (Vitis) gongylodes*. Simultaneously with the dissolution of the cell walls, the nuclei and nucleoli within the syncytia become significantly enlarged. The cytoplasm becomes dense with numerous small vacuoles in place of the single vacuole commonly found in normal cells. These cells are rich in storage material and serve to nourish the larvae in much the same manner as do the nutritive cells of insect galls. Plant cells surrounding the body of the parasite also become enlarged but generally have one nucleus and one vacuole, as do normal cell types. It appears, therefore, that syncytial formation and hypertrophy involve different phenomena.

In a series of papers Owens and his collaborators (71–73, 80) have studied in detail biochemical changes that are induced in host tissues by the root-knot nematode. Work done in this area has been reviewed by Krusberg (39). The results obtained in these studies tend to demonstrate that the rates of intermediary metabolism are accelerated in the galls especially in pathways leading to the synthesis of nucleic acids and proteins. It was shown that the developing syncytium is a region of intense ribo- and deoxyribonucleic acid synthesis. Incorporation patterns for uridine and thymidine were found to be dependent upon the developmental stage of the parasite as well as of the syncytium. DNA synthesis within a syncytium was found to be dependent upon the close association of the feeding nematode whereas RNA synthesis, once initiated, is apparently independent of the parasite. Dropkin (27) has
shown a high positive correlation to exist between gall area and numbers of larvae in a gall. He 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 and specialized growth, the development of which is dependent upon continued stimulation by the pathogen.

B. NON-SELF-LIMITING OVERGROWTHS

1. Crown Gall

The non-self-limiting tumors of a transplantable type are of interest because the new growth is composed of persistently altered, more or less randomly proliferating cells that reproduce true to type and against the growth of which there is no adequate control mechanism in a host. The affected cells thus acquire, as a result of their transformation, a capacity for autonomous growth which permits them to direct their own activities largely irrespective of the laws that govern so precisely the growth of all normal cells within an organism. Theoretically, the acquisition of the capacity of a cell for autonomous growth requires something newly activated and distinctive, something that urges such cell types to continued abnormal and essentially unregulated proliferation. The question as to what makes such tumor cell types unresponsive to the normal control mechanisms is fundamental and constitutes the ultimate basis of the tumor problem generally.

The tumor problem is basically a problem of growth. A fully autonomous rapidly growing tumor cell type may, in fact, be described as a highly efficient proliferating system the energy of which is directed largely toward a synthesis of substances required specifically for cell growth and division. The transition from a normal cell to a tumor cell must, therefore, involve a radical reorientation of biosynthetic activities going from the precisely regulated metabolism concerned with differentiated function, which is characteristic of a normal resting cell, to one involving the permanently increased synthesis of the nucleic acids, mitotic proteins, and other substances required specifically for cell growth and division. It would appear, therefore, that an understanding of the tumor problem is concerned with the characterization at a biochemical level of the cellular mechanism that is responsible for this major permanent switch in biosynthetic metabolism. In considering that

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question most of the results to be discussed below were obtained with the use as the experimental test object of the non-self-limiting tumorous disease of plants commonly known as crown gall. This disease is initiated by a tumorigenic principle (TIP) elaborated by the specific bacterium *Agrobacterium tumefaciens*. Other diseases of similar type, such as Black's wound tumor disease which is caused by a typical virus and the Kostoff genetic tumors which regularly occur as a result of irritation in certain interspecific hybrids within the genus *Nicotiana*, will be considered only insofar as they may contribute to an understanding of certain of the concepts that will be developed here. These diseases have recently been reviewed in detail (1, 5, 16, 17, 21, 23, 33, 36).

a. Tumor inception. i. Biology. Plant neoplasms, like the animal tumors, may be initiated by physical, chemical, and biological agencies. The implication of such findings would appear to be that all affect a common cellular mechanism which, once deranged, urges the cells to continued abnormal growth. This metabolic derangement could in all instances be a reflection of a single type of heritable change or it could conceivably result from any one of several possible types of nuclear or extranuclear modifications. The effectiveness of such agents in eliciting tumor formation appears, moreover, to be determined in large part by the hereditary constitution of the cells upon which such agents act. These tumorigenic agencies, with the possible exception of certain of the viruses, appear to be concerned only with the inception of a tumor and play no role in the continued abnormal and autonomous proliferation of the tumor cell once the cellular transformation has been accomplished. It is therefore necessary in any comprehensive discussion of the tumor problem to distinguish between the proximate cause which renders the cells neoplastic and the continuing cause or causes that are responsible for the continued autonomous growth of the tumor cell. Two stages are now recognized in tumor formation in the crown gall disease (18). The first of these is concerned with the transformation of a normal plant cell to a tumor cell; the second, with the continued autonomous proliferation of transformed cells into a neoplastic growth. In subsequent studies (9) two independent events, which have been termed conditioning and induction, have been found to be essential to complete the first or inception phase.

It had long been recognized that irritation accompanying a wound is necessary if tumors are to form in the crown gall disease. The reason for this remained obscure for many years. The necessity of a wound for tumor inception in crown gall was initially conceived of in terms of an entrance site for the bacteria (88), since it was believed that the inciting organisms were intracellular. This interpretation was ques-
tioned as a result of subsequent studies by others (78). A specific role for a wound in the inception process in the crown gall disease was reported (9) within the past decade. It was shown in those studies that host cells must be conditioned or rendered susceptible to transformation by irritation accompanying a wound if they are to be transformed to tumor cells. This effect was demonstrated by comparing the response of wounded tissue that had been permitted to heal for 48 hours prior to inoculation with the bacteria with the response obtained when the bacteria were inoculated directly into previously unwounded tissue. In both instances the bacteria were allowed to act for only 24 hours at 25°C, a period which is not in itself sufficient to permit the cellular transformation to occur when the bacteria are introduced into previously unwounded tissues. In those studies it was found that plants that were wounded 2 days prior to inoculation developed large tumors, while those inoculated at the time of wounding showed no tumorous response. The conditioning process was found to take place gradually, the cells reaching a maximum susceptibility to transformation between the second and third days after a wound is made, while predisposition of the cells to transformation declined again as wound healing progressed toward completion. For example, in Kalanchoe daigremontiana or Catharanthus roseus (Vinca rosea), tumors are not initiated in the first 24 hours or for the most part subsequent to the fifth day following inoculation of the plants with the bacteria. Very small slowly growing tumors are initiated either in 30–34 hours or at the 90–96-hour period following wounding. Moderately fast-growing tumors originate when the cellular transformation occurs at either the 40th hour or 80th hour, while the optimal period for initiation of tumors of the most rapidly growing type is between the 60th and 72nd hours after wounding. It is at that period in the normal wound healing cycle that large numbers of cell divisions are first observed (19). It is thus just before active cell division occurs in the wound healing cycle that normal cells are converted into tumor cells of the most rapidly growing type. Lipetz (53, 54) studied the effect of temperature on conditioning and wound healing in intact plants. He found that in the temperature range of 25°C–36°C maximum competence occurs earlier at the higher temperatures and that the temporal duration of the population of competent cells is inversely proportional to temperature.

Lippincott and Lippincott (56) reported that when pinto bean (Phaseolus vulgaris) leaves are infected with bacteria the number of competent cells converted into tumor cells increases linearly with time. It was suggested, moreover, that the conversion of cells in primary
pinto bean leaves into tumor cells is a single particle event at greenhouse temperatures (55).

Of interest are studies which demonstrate a striking increase in DNA and protein during the conditioning period. The rise in DNA, which represented an increase of 30–40%, was reached 48 hours after a wound was made and then declined abruptly, reaching control levels in a 4-day period. These findings were initially interpreted to indicate that the observed increased DNA was TIP itself (35). More recent studies, however, have demonstrated the rise in DNA to be the consequence of wounding and to occur entirely independently of the bacteria and, hence, of the TIP (43). The role, if any, of this DNA rise in predisposing the cells to transformation during the conditioning period is not clear at this time. If, however, the plant cells are not adequately conditioned, as appears to be the case in the early and late stages of the wound healing process as well as in normal cells not under the influence of a wound, the cellular transformation will not occur despite the fact that many virulent bacteria are in intimate contact with such cells. Predisposition of the host cells to transformation is, then, a transient character, the nature of which has not yet been elucidated.

It would appear that the reorientation in metabolism from that found in a resting cell to that present in a dividing cell is important to the transformation process. It may be that the cellular systems that are directly affected by TIP are exposed and thus rendered vulnerable to transformation in cells that are preparing to divide as a result of irritation accompanying a wound. Once transformed to tumor cells, they are prevented from returning to quiescence as they would during the normal course of wound healing. The pattern of synthesis found in a dividing cell thus appears to be perpetuated indefinitely in the tumor cell. The nature of such heritable changes will be considered later in the discussion.

The role of irritation accompanying a wound, although imperative for the initiation of tumorous growth in the case of the Kostoff genetic tumors and in Black’s virus tumor disease, appears to be less specific than it is in the crown gall disease. In those two instances the potential tumor cells do not develop into a neoplastic growth unless such cells are first stimulated to divide as a result of some relatively nonspecific irritant, such as wounding, application of hormones, radiation. The primary tumorigenic agency does not appear, in those instances, to induce quiescent cells to divide but, rather, the induced tumorous state prevents cells stimulated to divide as a result of irritation from returning to quiescence as they normally would.
ii. Histochemistry. Although the nature of the conditioning process remains uncharacterized, cytological and cytochemical studies have led to interesting findings following the transformation of such conditioned plant cells into crown gall tumor cells by the TIP associated with that disease. Prospective tumor cells in the broad bean (Vicia faba) could first be recognized 24–48 hours after inoculation by marked increases in nuclear and nucleolar volumes that were significantly greater than those found in wounded control stems (76, 103). It was found, further, that in cells containing constant levels of DNA the histone protein was not quantitatively altered as a result of the cellular transformation, but that the amounts of both nucleolar and cytoplasmic RNA and non-histone protein increase significantly by the third day after inoculation over that found in the wounded controls (76). This would appear to reflect a very early activation of mechanisms concerned in protein synthesis in the incipient tumor cells. Whether such changes are causal or merely secondary effects of more fundamental changes that result during the transformation process is not yet entirely clear (75).

b. Tumor development. i. Physiology. Activation of essential biosynthetic systems. Following the transformation of normal plant cells to crown gall tumor cells, the continued abnormal proliferation of the affected cells becomes an automatic process that is entirely independent of any recognizable infectious agent. This aspect of the tumor problem is concerned specifically with growth, and insight into that area would appear to depend largely on an understanding of the physiological and biochemical processes underlying normal cell growth and division. These fundamental growth processes appear to be dependent for their development in higher plant species upon the quantitative interaction of two growth-regulating substances, the auxins and the cytokinins. The auxins are concerned with cell enlargement, while the cytokinins are mitogenic and act synergistically with the auxins to promote growth accompanied by cell division (32, 96).

It has long been recognized that auxin plays a central role in the establishment of growth autonomy in the crown gall tumor cell. Early observations (57) indicated that certain plant species such as the tomato (Lycopersicon esculentum) respond to infection by virulent crown gall bacteria not only with tumor formation, but also with an epinasty of the leaf petioles, initiation of adventitious roots, inhibition of lateral buds, delayed abscission of senescent leaves, etc., all of which suggest that excessive amounts of auxin are synthesized by the developing tumors. Such responses were much less pronounced or entirely absent in plants similarly inoculated with attenuated strains of the inciting bacterium.
The finding some years later (22, 109) that sterile crown gall tumor tissues grow profusely and indefinitely on a simple inorganic salts-sucrose-containing culture medium that does not support the growth of many normal cell types in the absence of an exogenous source of auxin added credence to the belief that crown gall tumor cells synthesize auxin in significant amounts. Other lines of evidence soon appeared which, although of an indirect type, suggested that the rate of growth of a tumor was, in part at least, a function of the amount of auxin synthesized by the cells of the tumor (18, 106). That an auxin can be isolated from crown gall tumor tissues is evidenced from the studies of Kulescha (40), Nitsch and Nitsch (69), and Nitsch (70). That it is required specifically for the growth of the tumor cells is suggested by studies in which antimetabolites were used (38).

Similarly, the evidence appears now to be conclusive that cytokinins are synthesized by and are required for the continued growth of the crown gall tumor cell. The synergistic effect of auxins and cytokinins in promoting cell division was first suggested by the studies of Steward and Caplin (96). In that same year Skoog and Tsui (87) reported that tobacco pith parenchyma cells enlarged greatly in size in the presence of an auxin, without, however, dividing. It was later found (32) that such cell types could be made to grow and divide actively if they were treated with both an auxin and coconut milk. Shantz and Steward (83) demonstrated a number of different biologically active substances in coconut milk capable of promoting cell division in carrot phloem tissue. One of these was identified as 1,3-diphenylurea (84). Substances possessing comparable biological activity have now been found in many different plant tissues. Miller (61) and Miller et al. (62, 63), showed, moreover, that 6-furfurylaminopurine is highly active in promoting cell division in tobacco pith parenchyma cells when used in association with an auxin. That substance, which was synthesized and given the trivial name of kinetin, was initially isolated from heated or aged solutions of herring sperm DNA. It does not appear to occur naturally in plants.

Extracts of crown gall tumor tissue have been shown to be a rich source of substances of a cytokinin type which is limiting for cell division in normal tobacco pith tissue (20) as well as in certain other kinds of plant tissues (97). Two cytokinins have recently been isolated in pure form and in part chemically characterized from crown gall tumor cells of Catharanthus roseus (Vinca rosea). These substances have been found to be very similar in their chemical, physical, and biological properties. They both contain nicotinamide, a glucose sugar moiety, sulfur in the form of sulfate or sulfonate, one or more methyl groups, and at
least one straight-chain fatty acid. These substances show biological activity in a tobacco pith assay at a concentration of 50 µg per liter (110). It is interesting to note, moreover, that normal *Catharanthus roseus* cells stimulated to grow with kinetin synthesize a cytokinin that has physical, chemical, and biological properties that are very similar to if not identical with the tumor cytokinins. These results suggest that kinetin may not itself be a mitotic triggering agent, as is now commonly believed, but rather that it may act indirectly to induce the synthesis by normal cell types of a naturally occurring cytokinin (113).

Studies of the type reported above clearly demonstrate, then, that two growth factors, one of which, an auxin, is concerned with cell enlargement while the other, a cytokinin, promotes cell division when used in association with an auxin, are essential if growth accompanied by cell division is to occur in normal tobacco pith parenchyma cells. The pith cells have apparently lost, as a result of their maturation, the capacity to produce physiologically effective concentrations of those two growth substances. Since both growth substance-synthesizing systems appear to be solidly blocked in the tobacco pith parenchyma cells, an attempt was made to learn how such cell types would respond when transformed into crown gall tumor cells (11). If, for example, only the auxin-synthesizing system is activated as a result of the transformation of normal cells to tumor cells, then the altered pith cells should enlarge greatly in size but should not divide. If, on the other hand, the cytokinin-synthesizing system is activated without a corresponding activation of the auxin system, then neoplastic growth should not result because, as indicated above, a cytokinin without auxin is ineffective in promoting growth in pith parenchyma cells. Only if both growth substance-synthesizing systems are permanently activated during the transformation of normal cells to tumor cells will a tumor develop in this test system. The results of that study clearly demonstrated that when healing pith parenchyma cells were transformed to crown gall tumor cells a typical crown gall tumor developed. Thus, although normal tobacco pith cells could not synthesize either an auxin or a cytokinin, after their transformation to tumor cells both substances were produced in greater than regulatory amounts. If that were not true, continued growth accompanied by cell division and, hence, tumor formation would not have resulted in the test system used in that work.

That those two growth-promoting substances were, in fact, actively synthesized by growing tumor tissue was demonstrated by grafting a fragment of sterile tobacco tumor tissue on a fragment of normal pith parenchyma tissue. As the tumor tissue grew, the normal pith parenchyma cells were stimulated to very active division without, however,
being transformed into tumor cells. It is clear, therefore, that the tumor cells synthesize greater than regulatory amounts of an auxin and a cytokinin. The permanent activation of those two biosynthetic systems with the resulting production of excessive amounts of the growth-promoting substances would appear in itself to be sufficient to account for the continued abnormal and autonomous proliferation of the crown gall tumor cell. Subsequent studies revealed, however, that not only those two but other biosynthetic systems, which represent the entire area of metabolism concerned with cell growth and division, become progressively and permanently activated during the transition from a normal plant cell to a fully autonomous crown gall tumor cell (13).

An experiment such as that described above gives a great deal of information concerning the workings of this system. It demonstrates that as a result of the transition from a normal cell to a fully autonomous tumor cell a series of quite distinct but well defined biosynthetic systems which represent the entire area of metabolism concerned with cell growth and division become progressively and persistently activated. The degree of activation of those systems determines, moreover, the rate of growth of a crown gall tumor cell.

This study demonstrates, furthermore, why a plant tumor cell grows autonomously. Autonomy, in this instance, finds its explanation in terms of cellular nutrition. The tumor cells have acquired the capacity to synthesize all the growth factors that their normal counterparts require but cannot make for cell growth and division.

Finally, this study shows that as a result of the transition from a normal cell to a tumor cell a profound and persistent switch in the pattern of synthesis occurs, going from the precisely regulated metabolism which is concerned with differentiated function and which is characteristic of a normal resting cell to one involving the persistently increased synthesis of the nucleic acids, the specialized mitotic proteins and other substances required specifically for cell growth and division. This new pattern of synthesis is triggered by irritation accompanying a wound. It is maintained in the crown gall tumor cell by virtue of the fact that that cell type has acquired the capacity to synthesize continuously the two hormones that regulate cell growth and division. The other metabolites shown to be produced by the tumor cells are required for the continued production of the nucleic acids, the structural and enzymatic proteins and, in the case of myo-inositol, the membrane systems of the cell. It is those metabolites that permit the pattern of synthesis concerned with cell growth and division to be expressed.

It is interesting to note that in certain other non-self-limiting tumor-
ous diseases we find that, in the case of the Kostoff genetic tumors, the tumor tissue grows well on a simple chemically defined culture medium that does not support the growth of normal cells of either parent. Similar findings have been reported in the case of Black's wound tumor disease, which is of viral etiology. Thus, in these instances, as in crown gall, the tumor cell acquires a capacity to synthesize all the factors needed for continued cell growth and division.

It is, therefore, clear from the results of studies reported above that, although three different and quite distinct agents can initiate the tumorous state in plants, the physiological basis for autonomous growth appears to be similar in all three instances.

If the concept of cellular autonomy involving the permanent activation of biosynthetic systems outlined above is correct in its essential features, it should be possible to reproduce, superficially at least, in vitro not only the morphological but also the histological and cytological abnormalities that characterize the tumorous state in crown gall with the use of certain normal cell types as an experimental test object.

It is clear from an abundant literature on the subject that crown gall tumors may show differences in their morphology depending upon the host species in which they develop. The character of the resulting tumor appears to depend in large measure on three factors (10). The first of these appears to reflect the degree of cellular transformation achieved during the inception period. High degrees of cellular transformation commonly result in tumors that grow very rapidly and cause a pronounced disruption of the normal growth pattern of the host tissue in which they develop. Such tumors are highly disorganized histologically, may be poorly vascularized and have no characteristic size or shape. Because of inadequate vascularization they may become necrotic. Tissue cultures of sterile tumor tissues of this type grow very rapidly, are friable, and the cells hang loosely together. In contrast, cells that possess low grades of cellular change grow very slowly and remain localized in a host. Such tumors have a smooth external contour, may be covered with a layer of epidermal cells, and show a higher degree of cellular differentiation than do the fully transformed tumor cells described above. Cells from such tumors grow slowly and compactly in a simple inorganic salts-sucrose-containing culture medium.

A second factor that determines the morphological character of the tumor is concerned with the potentialities of the host cells for regeneration at the time that the cellular transformation is accomplished. Cells of plant species that possess low competence for regeneration develop into typical unorganized tumors regardless of the degree of alteration
achieved at the time of the cellular transformation. When, on the other hand, pluripotential cells that possess highly developed regenerative competencies at the time of their alteration are transformed to tumor cells, the character of the resulting tumor is determined by several factors. This may be illustrated by the following type of experiment.

When a plant such as tobacco, the cells of which possess highly developed regenerative competencies, is cut through an internode at about the middle of the plant and the cells at both cut surfaces are transformed to tumor cells by a TIP elaborated by a moderately virulent strain of the crown gall bacteria, typical unorganized tumors develop at the basal end of the upper cutting. The other inoculated cut surface, which has become the tip of the lower half of the plant, develops into a complex tumor or teratoma. Such teratomata are composed in part of a chaotic assembly of morphologically highly disorganized tissues and organs. The cells present at the two cut surfaces prior to the time of their separation possessed the same potentialities since they were adjoining cells in the same stem. Immediately after the cut was made, however, the cells below the point at which the stems had been severed became the apical cells of the basal portion of the original plant, while the cells above the cut became the basal portion of the upper cutting. Sterile tumor tissue fragments isolated either from teratomata initiated at the cut stem tips or from the unorganized tumors that developed at the basal end of the tobacco cuttings were similar in growth pattern when cultivated in vitro. Cells isolated from either type grew profusely on a simple chemically defined culture medium and retained indefinitely a capacity to organize morphologically highly abnormal leaves and buds. Thus, although the sterile tissue fragments were isolated from two morphologically very distinct tumors, their growth patterns in culture were similar. Such sterile fragments, when grafted to the cut stem tips of tobacco plants, developed into typical teratomata. Similar fragments implanted into internodes of tobacco plants containing functional apical buds developed into crown gall tumors of the typical unorganized type. The restraining influence that a host containing a functional apical bud exercises on the organizational capacity of such pluripotent tumor tissues is doubtless hormonal in nature. These results illustrate, then, that the ability of pluripotent tobacco tumor cells to organize and develop into teratoma is a function of the position that they occupy in the plant axis. When such pluripotential tobacco cells are transformed to tumor cells by a TIP associated with a highly virulent strain of the inciting bacteria, they lose permanently the capac-
ity to organize morphologically abnormal structures, indicating that the cellular factors concerned with differentiation and organization are completely overwhelmed by the effects of such a TIP.

Cells of a plant species such as *Catharanthus roseus* (*Vinca rosea*), which do not possess the regenerative competencies of pluripotential tobacco cells, are altered to tumor cells of a type incapable of organization by both a TIP-associated with a highly virulent and a moderately virulent strain of the crown gall bacteria. Thus, although the TIP elaborated by a moderately virulent strain is incapable of canceling the organizational capacity of pluripotential tobacco cells, that principle does initiate the development of unorganized tumors when plant cells possessing low competency for regeneration are transformed to tumor cells by it. Therefore, three factors, (a) the degree of cellular transformation achieved, (b) the position that the tumors occupy in the plant axis, and (c) the competency of cells for regeneration, are all concerned in determining the morphology of a crown gall tumor.

Histological examinations of typical crown gall tumors commonly show a highly disorganized mass of small rapidly dividing cells and very large single or multinucleate giant cells interspersed among poorly organized groups of vascular elements. Polyploidy and polyteny are characteristic of many such tumors, although Kupila (42) has reported that tumors in the sunflower (*Helianthus annuus*) are wholly diploid with cells of uniform size.

The morphological as well as the abnormal histological and cytological picture observed in crown gall tumor tissue can be accounted for in terms of the abnormal growth substance physiology which is characteristic of such tissues. It has been possible to reproduce, under defined experimental conditions and with the use of certain normal cell types as the experimental test object, the several morphological growth patterns which include rapid friable and slow compact growth of the unorganized type as well as teratoma-like structures (12, 20).

In those studies a correlation was found to exist between the concentration of an auxin in an otherwise suitable culture medium containing a constant level of a cytokinin and the amount and rate of growth that occurred in normal tobacco pith parenchyma tissue fragments. When relatively high levels of an auxin of the order of 1 mg of naphthalene acetic acid per liter were incorporated into a culture medium containing a constant level of cytokinin, the normal pith fragments grew very rapidly and the tissues were friable. The growth pattern of such tissues showed a striking resemblance to crown gall tumor tissue of the most rapidly growing type. When the auxin level was reduced to 0.05 mg per liter in the cytokinin-containing medium, the
normal tobacco pith cells grew slowly and in a compact manner. They resembled the growth pattern when low grades of neoplastic change are achieved in the crown gall disease. Further reduction of the auxin level to 0.01 mg per liter in the cytokinin-containing culture medium resulted in the very slow and unorganized growth of the pith parenchyma cells for a period of 3–4 weeks. Thereafter, numerous more or less organized structures appeared at the surface of some of the pith tissue fragments. Such tissues showed a superficial resemblance to crown gall teratoma tissue of tobacco. It is clear, therefore, that a balance of auxins and cytokinins controls not only the processes of cell enlargement and cell division but also organization and cellular differentiation as well, a concept that has been so elegantly developed by Skoog (85) and Skoog and Miller (86).

Studies such as these demonstrate, then, that it is possible to reproduce under controlled experimental conditions growth patterns that resemble the morphologically distinct types of crown gall tumors that have thus far been described as occurring in tobacco. The artificially stimulated normal pith tissues are, however, self-limiting growths, and when the externally supplied stimuli are removed their growth promptly stops. They are growth substance-stimulated hyperplasias. Crown gall tumor tissue is, on the other hand, autonomous and is itself capable of synthesizing all the growth factors required for its continued abnormal proliferation. The characteristic histological picture involving hyperplasia and hypertrophy leading to disorganization and loss of function as well as cytological abnormalities such as aberrant nuclear behavior, polyploidy, polyteny, single or multinucleate giant cells, etc., that characterize the tumorous state in crown gall have also been reproduced under defined conditions. The histological and cytological abnormalities appear also to reflect the abnormal growth hormone physiology of the tumor tissues.

Patau et al. (74) have suggested that auxin is necessary for DNA doubling as well as for mitosis, whereas a cytokinin is required for mitosis and cell division and possibly also for DNA doubling. Because of the abnormal hormonal relations found in plant tumors, a shift in the pattern of DNA synthesis and mitosis might be expected during tumor growth. This could and probably does lead either to the production of large nuclei of high DNA content or to the progressive reduction of large nuclei once they have formed. The fact that such artificially stimulated normal cells commonly show histological and cytological characteristics of 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.
ii. Biochemistry. (a) Respiration. Many studies have been made comparing the rates of respiration in normal and crown gall tumor tissues of plants. Neish and Hibbert (66), for example, reported the respiratory quotient of normal and tumor tissue of the sugar beet to be 0.64 and 0.92, respectively. The low R.Q. of the normal tissue was explained by the formation of citric, malic, and oxalic acids. It was found that the amount of these three acids accounted for about 75% of the oxygen consumed in excess of that required for an R.Q. of unity. Since such acid formation did not occur in the tumor tissue, the experiments suggest a marked difference in metabolism of the two types of tissue. Eberts et al. (28) reported that the addition of certain organic acids increased significantly the rates of oxygen uptake in tomato tumor and normal tissue slices. These workers reported, further, that the $Q_{O_2}(N)$ (microliters of oxygen consumed per milligram of tissue nitrogen per hour) of exhaustively washed slices of tumor tissue of tomato was lower than that of healthy stem slices similarly treated. Klein (34) obtained similar results only when old tumor tissues were used. White (108) compared the respiratory behavior of a number of sterile tumor tissues of the sunflower (*Helianthus annuus*) with normal tissues. It was concluded from those studies that the two tissue types did not show significant qualitative differences in respiration, but a definite lowering of respiratory levels was observed in the tumor tissues. It was pointed out, however, that those conclusions, which were based on dry weight, may be misleading. Link and Goddard (50) compared on a wet weight basis tumor and normal tissue of tomato and concluded that the rates of oxygen uptake of tumor tissue slices were at all stages of development of the tumor greater than those found in the normal controls. Klein's (34) findings are in essential agreement with those of Link and Goddard except during the inception period, at which time Klein found a somewhat lower oxygen uptake in the tumor tissues. Berthelot and Amoureux (3) suggested, on the basis of their studies, that aerobic fermentation occurs in beet tumors. It is clear that differences in respiration of tumor and normal tissue must be based on some constant cellular character if observed differences are to be significant. Scott *et al.* (81) have recently examined that question and concluded that if results are expressed in terms of cell numbers, DNA, and protein, the normal and tumor cells of the red beet show no major differences in metabolic capacities, but that the main difference lies in the direction of cell metabolism. It was suggested that in the rapidly dividing tumor cells a greater proportion of the respiratory energy is utilized for protein synthesis. In the normal cells, on the other hand, much of the energy
derived from respiration is directed toward cell enlargement and the establishment of storage products.

Tamaoki and co-workers have recently reported the results of studies on the oxidative and phosphorylative activities of mitochondria from normal and crown gall tumor tissues of the tomato (99–102). The differences observed were slight quantitative, rather than qualitative, differences. Mitochondria isolated from such tissues were similar in their respiratory properties to those obtained from intact higher plants and animals, except for ascorbic acid oxidase activity. Mitochondria isolated from normal and crown gall tomato tissue cultures were assayed spectrophotometrically for their DPNH oxidase, DPNH-cytochrome c reductase, cytochrome c oxidase, and diaphorase activities. Normal mitochondria showed higher activities than did the tumor particles. There were, however, no qualitative differences in their catalytic properties. It was concluded from those studies that the two tissues had virtually identical pathways for the transport of electrons from DPNH to oxygen. Lance (46) has reported that mitochondria isolated from crown gall tumor tissue of Scorzonera possess twice the succinic and malic dehydrogenase activity per unit of protein nitrogen as does comparable normal tissue.

Brucker and Schmidt (25) studied the energy metabolism of normal and tumor tissue of Datura and carrot. It was concluded from those studies that a considerable portion of the energy of the tumor tissue is not derived from the Krebs cycle. These workers believe that in the normal cells terminal oxidation goes by way of the cytochrome–cytochrome oxidase system, while in the tumor the ascorbic acid–ascorbic acid oxidase system is involved. Lance (46) also reported that the cytochrome oxidase activity of the tumor tissue is always less than that found in corresponding normal tissue.

Spurr et al. (90) have recently studied the activities of ascorbic acid oxidase and tyrosinase in normal and crown gall tissues of the tomato at temperatures of 26°C and 32°C. A correlation was found to exist between tumor development and the activities of those two enzymes. Levi et al. (48) found tyrosinase and peroxidase activity far more active in the tumor tissue of the beet (Beta vulgaris) than in normal tissue. Ascorbic acid oxidase, on the other hand, was found by those investigators to be less active in tumor tissue. It was concluded that the increase in concentration of ascorbic acid in the tumor tissue was the result of a decreased activity of its oxidase. Studies based on cyanide-sensitive respiration indicated that the heavy metal enzyme systems mediate most if not all of the oxygen uptake of both normal and tumor
beet tissue. Link and Klein (51) reported that while iron, copper, and "residual" metalloprotein enzymes are each responsible for about one-third of the oxygen uptake of normal tomato stem tissues, the copper (tyrosinase) systems account for three-fourths of the oxygen uptake of old crown gall tumor tissues. Lance (45) found that an important part of respiration of both normal and crown gall tumor tissue of Scorzonera was insensitive to carbon monoxide inhibition.

Neish and Hibbert (66) attempted to account quantitatively for the total carbohydrate metabolized during a 3-hour period of respiration in tumor and normal beet root slices. In the tumor 25% of the sugar metabolized was accounted for by the CO$_2$ evolved, while the fate of the remainder was unknown. In the normal beet root tissue, on the other hand, the CO$_2$ evolved accounted for 19% of the sugar metabolized, while the combined oxalic, malic, and citric acids found represented a further 52%, the remaining 29% being unaccounted for. Under anaerobic conditions an alcoholic fermentation occurred in the tumor slices which accounted for 70% of the sugar metabolized. No detectable lactic acid production was observed although a small increase in keto acids was found. The metabolism of the normal tissue slices under similar conditions was quite different. Both alcoholic fermentation (19%) and lactic acid formation (24%) occurred. These account for almost 45% of the sugar metabolized under anaerobic conditions. The normal tissue but not the tumor tissue showed a decrease in the rate of carbohydrate catabolism when air was admitted to the fermenting tissues. Thus, the two tissues could be differentiated by the existence of a lactic acid fermentation and a Meyerhof effect in the normal but not in the tumor tissue. A pronounced Pasteur effect was found in both.

(b) Nitrogen metabolism. It is generally agreed that marked differences exist in the nitrogenous constituents of normal and crown gall tumor tissues (34, 37, 64, 67, 91, 98). It was found by Neish and Hibbert (67), for example, that, while about the same amount of nonprotein nitrogen exists in beet tumor and normal tissue, the tumors have a protein content about three times that found in the normal tissues. This increase in protein content was accompanied by a corresponding decrease in sugar content and it was suggested that the high level of sugar utilization by the tumor tissue indicates that the carbohydrates are being used not only for cell wall formation but for the specific synthesis of protein. Water-soluble protein was found, moreover, to be six times that present in the normal tissue. The tumors maintained 64% of their Kjeldahl nitrogen in the form of protein as compared with only 39% for normal tissue, thus reflecting a greater tendency of the tumors to synthesize protein.
Lee (47) made a comparative study of certain nitrogenous constituents present in crown gall, habituated, and normal tissue of the European grape (Vitis vinifera). No qualitative differences in the amino acid composition of these tissues was found. The crown gall tumor tissue had the highest concentration of total nitrogen and soluble nitrogen, while the normal tissue had the smallest amount of these constituents. Habituated tissue had intermediate levels. Lioret (52) made a comprehensive study of the amino acid composition of a number of species of tumor tissue as well as of normal tissue of salsify. Of particular interest in those studies was the finding of a new amino acid. This new amino acid was found to be present in large quantities in crown gall tumor tissue of salsify (Scorzonera hispanica), tobacco (Nicotiana tabacum), Virginia creeper (Parthenocissus quinquefolia [Ampelopsis hederacea]) and Jerusalem artichoke (Helianthus tuberosus) but it was not found in normal tissue of salsify. It was present in the tumor tissue only in the free state. This substance has now been isolated and found to be an analog of lysine (N-α-propionyl-L-lysine) which has been named lysopine (4). More recently, Seitz and Hochster (82) found the same amino acid to be present in normal tissues of Nicotiana tabacum and Lycopersicon esculentum although in significantly smaller amounts than in crown gall tumor tissues. No physiological function has been assigned to that substance.

(c) Phosphorus metabolism. Total phosphorus has been reported by a number of workers to be considerably higher in crown gall tumor tissue than in normal tissue (34, 47, 59, 65, 91). Phosphorus supplied as P\textsubscript{32} was found to accumulate far more extensively in the tumor tissue than in normal Kalanchoe pinnata (Bryophyllum calycinum) tissue (107). The concentration of total phosphorus was somewhat greater in the tumor tissue than in stem apices and much greater than in mature stem tissues. Klein (34) reported increases in phosphorus compounds in tomato tumor tissue as early as 5 days after inoculation. Orthophosphates and acid-soluble organic phosphates accounted for the observed increase. The total phosphorus content 14 days after inoculation was found to be twice that of the control and over half of this was due to an increase in the acid-soluble organic fraction. Harhash and Brucker (31) consider observed variations in the acid-soluble phosphorus fraction to be characteristic of crown gall tumor tissue.

Maciejewska-Potapczyk (58) found that the very high level of phosphorus in Datura stramonium tumor tissue results from increased amounts of acid-soluble phosphorus, lipid phosphorus, ribonucleic acid and deoxyribonucleic acid phosphorus. Scott et al. (81) recently reported that normal red beet tissue contained more inorganic phosphorus
than did tumor tissues when compared on either a wet weight or a DNA P basis. Both tissues contained comparable amounts of acid-labile P per unit fresh weight, though the normal tissues had about three times as much per microgram of DNA P. The approximate nucleotide content yielded results similar to those for acid-labile phosphorus. The residual lipid phosphorus was the same in both tissues when measured on a DNA P level but was found to be three times greater in the tumor tissue when measured on a per unit fresh weight basis. The RNA P content of the tumor tissues was significantly higher than that found in the normal. On a fresh weight basis, the RNA P content of the tumor tissue was four to five times greater than that found in normal, while on a DNA P basis the tumor tissue contained 1.4 times as much RNA P as did the normal tissues. These results confirm histochemical findings (76) and are of interest because the increased RNA and RNA: protein ratio of the tumor tissue tends to explain the higher rate of protein turnover in such cell types.

**c. Nature of the heritable change that leads to cellular autonomy.**

**i. Gene mutation versus epigenetic modifications.** Insight into the nature of the heritable change that results in the capacity of a tumor cell for autonomous growth is fundamental to an understanding of the basic mechanisms underlying tumorigenesis. If, for example, a heritable change in the phenotype can only occur following a change in the genotype, then tumorigenesis must involve somatic mutation at the nuclear gene level. If, on the other hand, such a change in the phenotype can be experimentally demonstrated to occur without a preceding change in the genotype, then quite different mechanisms can be postulated for the origin of a tumor cell. An attempt was made in the crown gall disease to distinguish between nuclear changes of a mutation type which involve the deletion, rearrangement or addition of genetic information and modifications of an epigenetic type that are concerned with changes in the expression of the genetic potentialities that are normally present in a cell.

As indicated above, fully autonomous crown gall tumor cells of the unorganized type are characterized by a capacity for rapid proliferation on a simple chemically defined culture medium, by a limited capacity for differentiation, and by a complete lack of an ability to organize recognizable structures such as roots, buds, or leaves. Since tumor cells of that type have now been maintained continuously in culture for more than a decade without showing the slightest tendency to become any less autonomous, they have generally been considered to be permanently altered cells. A second morphologically distinct type of crown gall tumor, the teratoma, is composed in part of a highly
chaotic assembly of tissues and organs. Such tumors arise, it should be
recalled, when pluripotent cells that possess highly developed regenera­
tive competencies are transformed to tumor cells by a TIP elaborated
by a moderately virulent but not by a highly virulent strain of the
crown gall bacterium. These teratomata, like typical crown gall tum­
ors, grow profusely and indefinitely on a simple chemically defined
culture medium that does not support the continued growth of normal
cells of the type from which the tumor cells were derived. They are
transplantable. That such teratoma tissue is composed entirely of tumor
cells, and not of a mixture of normal and tumor cells, was recently
demonstrated unequivocally by isolating a number of clones of single-
cell origin (14). Such single-cell clones behaved in every respect as
did the teratoma tissue from which they were derived.

One of the difficulties in gaining insight into the nature of the herita­
ble changes that lead to the autonomous growth of a tumor cell has
been the inability to reverse that process and effect a recovery of
such cell types under controlled experimental conditions. Plants have
been found to offer some very distinct advantages as experimental
test objects for studies of that type. The first of these deals with the
fact that somatic cells of certain plant species retain a high degree
of pluripotency which, as indicated above, is reflected in the formation
of teratomata with their morphologically abnormal leaves and buds.
The second advantage derives from the unique manner in which dicoty-
ledonous plant species grow. Primary growth in such plant species
results from the rapid division and subsequent elongation of the meris­
tematic cells at the extreme apex of a root or a shoot. The cells in the
meristematic region of a rapidly growing root or shoot divide at far
faster rates than do most tumor cells. It was, therefore, hypothesized
that if the highly abnormal tumor shoots derived from tumor buds
could be forced into rapid but organized growth, a recovery of the
tumor cells might possibly be accomplished regularly if the primary
cellular change leading to autonomy was concerned with a change in
the expression of the genetic potentialities, but not if it involved somatic
mutation at the nuclear gene level. The results of those studies clearly
demonstrated that when tumor shoots derived from tumor buds found
in teratomata of single cell origin were forced into rapid but organized
growth as a result of a series of graftings to healthy plants, they grad­
ually recovered and ultimately became normal in every respect (16a,
and references there cited). Recovery from the tumorous state was
complete. It was a gradual process that progressed in the direction
of the normal as the tumor shoots were forced into rapid but organized
growth by means of grafting techniques.
The results of studies described thus far indicate, then, that as the result of the transformation of a normal plant cell to a crown gall tumor cell, a profound switch in the pattern of synthesis occurs leading to the permanently increased synthesis of the nucleic acids, mitotic proteins, and other substances concerned specifically with cell growth and division. Studies on the recovery of the crown gall tumor cell demonstrate, moreover, that enzyme systems activated during transformation may again be blocked under certain special experimental conditions. This indicates that the area of metabolism concerned with differentiated function and which is characteristic of a normal resting cell is not irreversibly lost as a result of the transformation process but appears, rather, to be overwhelmed by the more primitive metabolism that characterizes the plant tumor cell. Since the nuclei of normal and tumor cell types appear to be genetically equivalent, the results of those experiments suggest that irreversible changes of a mutation type are not concerned in the transformation process in this system. The cellular transformation in this instance appears, rather, to be concerned merely with a change in the expression of the genetic potentialities that are normally present, but are somehow repressed, in normal cell types.

Smith (89) has recently suggested, from the results of his extensive studies on tumors that arise spontaneously in certain interspecific hybrids in the genus Nicotiana, that heritable changes similar to those described for crown gall may be involved in that system as well.

ii. **Regulation of biosynthetic systems.** The question that now arises is how are the biosynthetic systems concerned with cell growth and division regulated in normal and in crown gall tumor cells. It is clear that some very fundamental cellular mechanism must be involved in the simultaneous or perhaps sequential activation of the diverse biosynthetic systems shown to be unblocked in the crown gall tumor cell. Although the entire answer to that question is not yet available, some progress has been made. The results of those studies may be interpreted to indicate that five and in part six of the seven biosynthetic systems shown to be permanently unblocked in the plant tumor cell are either directly or indirectly ion-activatable systems (24, 111). Only the activation of the metabolic system concerned with the synthesis of the mitogenic substance, cytokinin, cannot as yet be accounted for on that basis.

The studies cited above, together with those reported in a recent paper (112), demonstrate that changes in the properties of the membrane systems do in fact accompany the transformation of a normal plant cell to a crown gall tumor cell. Such changes would appear to represent a most fundamental difference between a normal plant cell and a tumor cell since they permit the activation by ions of a large
segment of the metabolism concerned specifically with cell growth and division.

iii. The tumor-inducing principle. All the many attempts that have been made in the past to isolate and characterize chemically the tumor-inducing principle (TIP) that is responsible for the persistent heritable change that occurs in affected host cells have thus far been unsuccessful. This may have resulted from the fact that (a) TIP is very labile and is rapidly inactivated either thermally or enzymatically, or (b) that more than one factor is involved in the transformation process and that those factors must be introduced into a host in proper sequence to be effective, or (c) that the methods used for the reintroduction into plant cells of the TIP following its isolation have not been sufficiently refined to accomplish the cellular transformation.

From what is known of heritable cellular changes of the type being considered, it would appear reasonable to assume that the transformation process in crown gall results from the addition of new genetic information that is released by virulent strains of the inciting bacterium. There is, nevertheless, very little experimental evidence to substantiate such an assumption. It has recently been reported that ribonuclease A rather specifically inhibits tumor inception without affecting the capacity of either the bacterial cells or the host cells in the region of a wound from growing and dividing at a rate comparable to that of untreated cells. These results suggest either that the TIP or some essential component of that principle is a ribonucleic acid or that ribonuclease A enters the bacterial or host cells and in either case selectively inactivates some component essential for tumor inception without, however, affecting the capacity of such cells to grow and divide at a rate comparable to that of untreated cells (24a).

Evidence that new genetic information has been added during the transformation process in the crown gall disease might be obtained if it could be demonstrated that information resulted in the synthesis by the transformed cell of new and distinctive cellular components. As indicated earlier, five and in part six of the seven essential metabolites shown to be persistently synthesized by the crown gall tumor cell are also synthesized by the normal cell type if the concentration of four salts is raised in an otherwise suitable basic culture medium. This indicates that the information required for the production of those essential growth factors is present in the genome of the normal cell types. Only the activation of the cytokinin synthesizing system, which plays a central role in the development of a capacity for autonomous growth of the plant tumor cell, cannot as yet be accounted for on that basis.

Since the cytokinin isolated from Catharanthus roseus (Vinca rosea)
tumor tissue was found to contain a nicotinamide ring, a glucose sugar moiety as well as sulfur, it was very different in its chemical makeup from the 6-substituted purines that are generally regarded as being functional as cytokinins. Thus, the synthesis of this compound could have resulted from the presence of new genetic information that had been introduced into a cell at the time of transformation. Yet it was found (113) that when normal cells were supplied with an exogenous source of a 6-substituted purine such as kinetin, those cell types synthesized a cytokinin that was very similar in its physical, chemical, and biological properties to the cytokinin synthesized by the tumor cells in the absence of an exogenous source of a 6-substituted purine. Thus in this instance, as in the case of the other essential metabolites discussed above, the genetic information required for the synthesis of those compounds is present in the genome of the normal cell. Experimental results that may bear on this question were recently published by Reddi (77).

Reddi reported that a new and distinctive ribonuclease is present in fully transformed *Catharanthus roseus* (*Vinca rosea*) tumor cells that differs both quantitatively and qualitatively from the ribonuclease found in normal cells of that species. It also differs from the ribonuclease found in the bacterium. A correlation was found to exist, moreover, between the degree to which a cell had been transformed and the amount of the new enzyme present in a cell. On the basis of those results Reddi has suggested that this new enzyme may either have resulted from new genetic information that had been introduced into the cell at the time of transformation or that a segment of the normal genome which is ordinarily not functional and that is responsible for the synthesis of that enzyme had been derepressed. More work is required to distinguish between those possibilities.

The difficulty in deciding just how the addition of new genetic information is involved in causing a cell to grow abnormally as a tumor cell is evident in the case of Black's wound tumor disease. The causal virus in this disease has a diameter of about 60 μ and there appear to be 92 capsomeres forming the virus coat. The virus has an internal core of double-stranded RNA with a molecular weight of about 15 million. This represents perhaps 50 times as much nucleic acid as is needed by the virus to synthesize its protein coat. That is so even if just one of the two RNA strands is necessary for the coding of proteins. It is clear, therefore, that a significant amount of genetic information over and above that required for the synthesis of the protein coat is introduced into a plant cell during the infection process. It, nevertheless, seems quite unlikely that that new genetic information is concerned di-
rectly with synthesis of the essential metabolites including the auxins and the cytokinins, the continued production of which is required for the continued abnormal proliferation of the plant tumor cell. It seems more reasonable to assume that, as in the case of the crown gall disease, those regions of the host genome concerned specifically with cell growth and division are activated perhaps as a result of their persistent derepression by the virus. Just how this would be accomplished is not at all clear, but that very fundamental problem can now be approached experimentally.

References


