WOUND-TUMOR

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The wound-tumor disease of plants was discovered about thirty-five years after the isolation of the specific bacterial incitant involved in crown gall. Whereas the wound-tumor disease is of no known economic importance, crown gall is a serious disease of plants, and its ravages have provided a strong incentive for its study. Nevertheless, our greatest advances in understanding crown gall derive from investigations aimed to satisfy men's curiosity rather than from those designed to control the disease. By this time many biologists are familiar with crown gall; few are acquainted with wound tumor, although it is one of that interesting group of tumors incited by viruses.

Crown gall is chiefly a local infection with a limited development of secondary tumors in a few suscepts or hosts. Wound tumor, on the other hand, is caused by a systemic virus, and in most hosts it produces systemic irregular vein enlargement and many tumors on the roots. The wound-tumor virus has been transmitted to many plant hosts experimentally. It produces its most severe root-tumor manifestations in sweet clover, *Melilotus alba* Desr. and *M. officinalis* L. Lam., and in sorrel, *Rumex acetosa* L. Noticeable stem tumors occur only in sweet clover.

Histologically, the tumor contains meristematic tissue, sometimes organized as a cambium but usually consisting of scattered, meristematic zones. It also contains distorted tracheids and an abnormal phloem-like tissue. The tumor ordinarily contains no vessels and no sieve tubes. In the roots, most of the tumors originate in the pericycle; in the stems and leaves, the overgrowths start from the procambium, parenchyma, or fibers of the phleom. In roots of shepherd's purse, *Capsella bursapastoris* (L.) Medic., lateral roots are arranged in two rows directly opposite each other and running the length of the root; the initial tumor cells also form two rows precisely located on opposite sides of the root, midway between the points of origin of the lateral roots. In other words, at any time the point of origin of the next initial tumor cell can be predicted with great accuracy.

Tumors contain many spherical inclusions which have been shown to be rich in arginine. It is not known whether or not the inclusions are rich in virus.

Sweet-clover plants vary greatly in their hereditary response to infection with the virus, some producing many prominent root tumors and others very inconspicuous ones. Because it is a simple matter to produce sweet-clover plants from cuttings, many plants belonging to a single clone and having identical genetic constitution can be readily obtained. The virus can be introduced into a number of plants in each of several genetically different clones. Such a study has demonstrated clearly that the size, number, distribution, and morphology of the tumors on the stems of sweet-clover plants are affected by the heredity of the host.

There is abundant evidence that wounds are important in initiating tumors in virus-infected plants. About 80 per cent of the root tumors originate in the immediate vicinity of the emergence points of lateral roots. Lateral roots start in the pericycle and make wounds in the overlying endodermis, cortex, and epidermis through which they penetrate to the outside. On the stems of sweet clover, tumors are most common at the point of origin of branches, which are, of course, points of stress. They also occur at graft unions and at the cut surface of cuttings. Under optimum conditions they occur at artificially made wounds in young stems. The younger the wounded part of the virus-infected stem, the greater the probability that a tumor will develop from the wound. Tumors have also been associated with other injuries, such as those made by insects.

Nevertheless, it is not certain that wounds are essential to tumor development. In sweet clover, tumors frequently start in the pericycle lying between a legume nodule and the main vascular system of the root and, by their growth, cause the nodule to abort. There is no obvious wound present at this site.

It is supposed that some as yet unidentified wound hormone is important in mediating the effects of wounds. A synthetic growth-regulating substance, alpha-naphthalene acetic acid (NAA), when applied at 2 per cent concentration in lanolin to the epidermis, initiates a high proportion of tumors in the tips of virus-infected sweet-clover stems. Such stems contain many microscopic tumor initials composed of a limited number of cells. Apparently, most of these never develop into macroscopic tumors, but, within 72 hours after the application of NAA, proliferation of these microscopic tumors may be detected microscopically, and such growth ordinarily continues until a macroscopic overgrowth becomes obvious. Tumors stimulated to growth by the application of NAA are always associated with abortive adventitious roots. No synthetic growth hormone is known to stimulate the development of the microscopic tumors without the development of these abortive adventitious roots. Because such roots do not appear in association with normally occurring tumors, it seems certain that any hypothetical wound hormone playing a role in naturally occurring tumors must have a different composition from any of the synthetic growth regulators that have been tested. The application of the same materials to healthy stems stimulates adventitious root development but not tumors.

The wound-tumor virus is not transmissible by rubbing juice from diseased plants over the leaves of healthy plants. It is poorly transmissible when pin pricks are made through extracts from tumors rich in virus into the crowns of crimson clover plants. Only about 5 plants out of 100 are infected by the latter procedure.

In nature the virus is transmitted specifically by a few species of closely related leafhoppers, the most important being *Agallia constricta* Van Duzee and *Agalliopsis novella* (Say). Certain other closely related leafhoppers, such as *Aceratagallia sanguinolenta* (Provancher), are unable to transmit the virus. Virus may be detected in extracts by injecting small volumes (approximately 0.1 μl.) into the leafhoppers, which weigh about 1–2 mg. The virus must undergo an incubation period in the leafhopper before the insect is able to infect plants. Once the insect begins to infect plants, it may do so repeatedly over a period of several weeks. By the use of the injection technique, it was demonstrated that the tumors contain about one hundred times the concentration of virus present in the non-tumorous portion of the plant. The technique was also used to show that the virus can be diluted and transmitted from insect to insect repeatedly in series without loss of virus concentration in the insects, even though they are maintained on plants immune to the virus.

At optimum temperatures, that is, between 25° and 32° C., the minimum incubation period in the insect after the acquisition of the virus by feeding is about 2 weeks. The incubation period in the plant is of approximately the same duration. From 1 to 10 per cent of the progeny from infective females receive virus through the egg and are subsequently able to infect plants, even though they have had no chance to acquire virus from plants themselves. No symptoms of infection in the leafhoppers are known.

By means of zonal density-gradient centrifugation and electrophoresis, highly purified virus has been obtained from the plant tumors and also from the insect vector. In both cases, the virus proved to be a polyhedron about 75 mμ across.

Antisera of high titer (1/1,600) may be obtained by injection of the highly purified virus, suspended in a mineral-oil emulsion, into the muscles of rabbits. Antisera have been obtained by injection of virus purified from the plant tumors and, in another instance, purified from the insect vector. The antisera contain precipitating complement-fixing and neutralizing antibodies—the latter demonstrable in vector-injection experiments. The antisera do not react with plant protein or with insect protein in precipitin tests. Some tumor viruses of animals are claimed to incorporate host antigen. In the case of wound tumor the evidence indicates that this is not so.

In the infected plant and in the viruliferous insect, a soluble antigen related to the virus has been demonstrated. This subvirus is non-infective and does not occur in virus-free plants or insects.

Virus maintained in sweet clover for many years without access to the insect vector lost its ability to be transmitted by the insect. This vectorless virus is less virulent than the wild or vectorial virus. The former incites a smaller proportion of root tumors in relation to the total plant weight than does the vectorial virus. However, because the initial tumor reaction on the roots is less virulent, the plant makes better growth, and the total weight of tumors from the individual plant containing the vectorless virus is greater than it is from a corresponding plant containing the vectorial virus. Tumors incited by the vectorless virus contain approximately one-fourth the virus and four times the subvirus concentrations of those in tumors incited by vectorial virus.

Tumors incited on the roots of *Rumex acetosa* L. by the wound-tumor virus have been isolated and grown on a synthetic medium. This tumor tissue has an unusually high phosphate requirement and liberates an alpha-amylase into the medium in which it grows. Normal tissues grow very poorly or not at all in such a medium and do not liberate detectable amounts of the enzyme.

Sweet-clover tumors incited by the virus may be inoculated with crown-gall bacteria and will respond by the development of a superimposed crown-gall tumor. One cannot be certain that this indicates that the two agents—the virus and the bacterium—act on different cell systems to give tumefaction. The additive effects of cumulative periods of exposure to the crown-gall stimulus make this interpretation uncertain.

Wound tumor resembles virus tumors in animals not only because of the virus etiology but because of the important influence of the heredity of the host on tumor expression. It is perhaps particularly noteworthy that the virus, although it multiplies in the insect, appears to be completely innocuous therein. In sweet clover the virus incites the development of many microtumors which ordinarily do not attain a macroscopic development, which may be stimulated by the application of certain synthetic plant growth-regulating substances. It is possible that wounds act in a similar fashion through the agency of a hypothetical wound hormone. The virus apparently changes the physiology of the plant cell so that it is able to grow in a synthetic medium which does not support, or supports only poorly, the growth of normal tissues. There is also evidence that the tumor cell liberates alpha-amylase, the liberation of which cannot be detected from corresponding normal tissue. Cultured tumor tissue requires a higher phosphate concentration in the nutrient solution than does normal tissue.

An expanded account of information in this résumé is being prepared for Volume 15 of the *Handbuch der Pflanzenphysiologie* (Berlin: Springer-Verlag).

CONCLUSIONS

BY A. J. RIKER, *Chairman*

The men working with plant tumors have learned much from their associates on the animal side. While, of course, higher plants and animals are obviously different, still the basic activity of their cells is similar in many respects. This similarity was delightfully expressed many years ago by a biochemist. Let us consider the plant cell as one machine and the animal cell as another machine. Let us think, for example, of the amino acids, sugars, and vitamins as wheels in these machines. Then we see that many wheels are identical and interchangeable between the two machines. Many fundamental similarities are obvious. Only yesterday, Dr. Woolley explained the chemical relationships between serotonin in animal cells and indole-3-acetic acid in plant cells. These chemicals are related indole derivatives. They have comparable critical roles in the metabolism of cells where they occur.

The speakers today have contributed to fundamental biology. While the significance has been touched on in the different papers, perhaps for clarification I should mention a few of the trails being marked in this biological frontier for further basic research. For example:

1. Alternate pathways in nutrition and latent or unused enzyme systems are being clarified.

2. The induction of tumors is being accomplished by many different chemical, physical, and biological agents, including viruses. In certain cases the time of change from a normal to a tumor cell was determined with great precision.

3. The probability is being explored that certain tumors represent sectoral chimeras resulting from mutations of single cells.

4. A rather precise relation exists between the factors which are responsible for tumor-cell formation and the alteration in the synthetic capacity of the changed cells. The metabolism of the tumor cell is being studied in various ways to clarify how it is out of balance in comparison with that of the normal cell.

5. The development of tissue culture techniques permits the application to higher plant tissues, both diseased and normal, of the bacteriological and biochemical methods so fruitful both in microbiology and in studies of higher forms.