CROWN GALL

NOTES FOR STUDENTS

Crown gall.—Recent developments in the study of crown gall and its relation to animal cancer have been presented by Smith in several papers. The first of these, in point of completion although not in time of publication, is a succinct account of remarkable growth phenomena resulting from the action of Bacterium tumefaciens when inoculated into special tissues of plants. Four cases are distinguished.

1. When the internodal cambium is inoculated, this tissue loses its tendency to form mature structures having definite orientation. Instead, the cells continue to divide rapidly, forming large masses of mostly embryonic parenchyma within which scattered and irregularly arranged xylem and phloem elements are differentiated. The process recalls that described by Lamarlière in the galls of Gymnosporangium and aptly designated by him as "parenchymatization."

2. When the cortical parenchyma is infected, a somewhat similar development takes place. The cell divisions succeed each other so rapidly that the cells in the proliferating tissue remain small in comparison with the normal parenchyma, and appear to remain continually in an embryonic state. In time, however, there is a tendency to develop vascular elements, and these are then arranged in a more or less well defined stele. The vascular system of such tumors has no connection with that of the stem, consequently the galls soon die from imperfect nutrition and lack of water. The galls of these two types exhibit no external differentiation. They include all the forms of crown gall described in former papers.

3. A more remarkable condition is brought about when the crown gall organism is inoculated into the leaf axils of young growing plants (species of Pelargonium, Nicotiana, Lycopersicum, Citrus, and Ricinus). The tumors


thus produced are covered with abortive leafy shoots or with flower shoots if a flower incept has been disturbed. On tobacco plants these teratoid tumors may give rise to secondary tumors similar in nature. These daughter tumors are connected with the parent growths by tumor strands which are quite different in structure and location from those occurring in galls of the first two classes. The tumor strands heretofore described were found in the Paris daisy. They arise in the region of the primary xylem and consist of parenchyma tissue. The new tumor strand found in the tobacco occurs in the cortex. It consists of a concentric bundle with the xylem surrounded by the phloem. The daughter tumors arise at intervals along the strand and often have all the characteristics of the parent tumor.

4. The last case, even more remarkable, results when the young leaves of tobacco plants are infected with the crown gall organism. From such infections on the midrib and lateral veins tumors arise which produce leafy shoots. These tumors the author regards as akin to teratoid tumors in animals. The fact of their development is another proof that any plant cell not fully matured may retain the capacity for developing the whole organism.

In another paper, written for medical readers, the subject of crown gall is discussed in its relation to the problems of human cancer. The general resemblances in mode of growth, cell multiplication, occurrence of tumor strands, and production of secondary tumors in the two classes of growths are pointed out. The materials presented in this paper are essentially those of earlier papers, together with the new facts of the paper reviewed above. The phenomena, however, are described in greater detail, and considered with special reference to their bearing on animal pathology. Here, as in other cases, the author relies mostly on numerous excellent photographs for the presentation of his evidence.

In explaining his standpoint with reference to the bearing of his work on the problems of animal cancer, the author makes no claim that the causal organism of the crown gall has any relation to human cancer. It is pointed out, however, that this organism induces in plants a set of phenomena which have a striking parallel in the manifestations of animal cancer. Such phenomena are the growth without function shown by gall tissue, the persistently embryonic character of the proliferating cells, the lack of orderly differentiation of the tumor tissues, the existence of tumor strands giving rise to daughter tumors repeating the structure of the parent gall, and the occurrence of galls resembling embryonic teratoids. It is further pointed out that in the crown gall the cell, although apparently possessing invasive capacity, is not itself the parasite, as


5 It appears that the embryonic tissue of the gall to a certain extent pushes in among the cells of the sound tissue, a phenomenon which distinguishes this growth from other plant galls induced by fungous or animal parasites. The mode of progress
Jensen thought. On the contrary, the behavior of the cell is due to an invading specific microorganism. These facts, together with the observation that in one case at least (Rous' sarcoma of fowls) the abnormal growth can be produced by some sort of material separable from the cells and capable of multiplying when injected into other tissues, are regarded by the author as greatly advancing the contention that animal cancer is due to an intracellular parasite.

Exceedingly interesting results were obtained in a series of experiments designed to determine the more immediate causes of tumor growth in crown gall. In this investigation the author was guided by the hypothesis that the substances produced in the metabolism of Bacterium tumefaciens must be the direct cause of the cell proliferations. To the end of determining the effects of such substances various plants were injected first with compounds which chemical studies had shown to be products of the causal organism, and finally with a large number of other substances.

The first experiments were conducted with ammonia, which in various concentrations was injected into the stem cavity of Ricinus and into the fruit cavities of young green tomatoes. The result of these injections was an abundant formation of cushion-like intumescences within the cavities in both cases. Later, proliferations of the same type were obtained by the injection of a large number of other substances, including ammonium salts of organic and of inorganic acids, dilute solutions of the acids themselves, salts, glucose, and saccharose, and in some instances to a slight extent with distilled water. In many cases when the tissues of the pith cavities of Ricinus were exposed to weak ammonia vapors from dilute solutions of ammonium phosphate or ammonium carbonate in tubes sealed into the hollow stems, proliferations were produced not only in the cavities containing the reagents but also in many internodes above and below the opened one. The action in these cases, therefore, took place at a distance through thick partitions. The most striking result was obtained from the injection of a 5 per cent solution of ammonium dihydrogen phosphate into a very young internode of Ricinus. In this instance the pith cavity became completely filled by the proliferating pith, and from this tissue a complete vascular cylinder was differentiated. The orientation of the new inner cylinder was the inverse of that of the normal cylinder, the phloem being at the center and the xylem occupying the outer region. Such a complete cylinder was observed only once, but in many instances isolated

of the tumor strand through the tissues is not yet clear. Whether this structure pushes its way through the pith or cortex by apical growth after the manner of the internal roots of lycopods, or whether progress through the tissues is accomplished by successive cell-invasion by the bacteria and subsequent differentiation of the invaded cells into the characteristic tumor strand, has not yet been determined. From study of his stained sections Smith thinks that both types of invasion occur.

concentric bundles were produced in the proliferating pith. In these the phloem was always at the center of the bundle. Such bundles, the author points out, occur normally in the axes of the inflorescence of *Ricinus* and in the nodes. Superficial intumescences similar to those reported by von Schrenk\(^7\) were produced on cauliflower by exposure of the plants to vapors of ammonia and of acetic acid mixed with alcohol.

The outgrowths here described all partake of the nature of intumescences frequently observed in plants. In some cases, indeed, as in the instance described of the complete filling of the pith cavity and the subsequent differentiation of a vascular ring, the outgrowth is excessive. This behavior leads the author to the belief that if the stimulus could be continually applied, one would have a condition resulting in the production of tissue masses not unlike those of crown galls. Since in his experiments the outgrowths also resulted from the presence of many substances not the product of parasites, the author is inclined not to attribute the effect to the specific chemical action of any compound, but seeks for an explanation in some property common to all the compounds regardless of chemical composition. Such a common characteristic he finds in their osmotic action, to which, rather than to chemical stimulation, he ascribes their effect. In this connection it is of interest to recall that intumescences have been produced by Atkinson,\(^8\) Miss Douglas,\(^9\) and Steiner\(^10\) by subjecting plants to conditions increasing water absorption and diminishing transpiration; and by Sorauer, Küster, von Schrenk, and others as a result of application of solutions. In the author's own work the intumescences were mostly the result of injection of solutions, but in some instances they resulted from the injection of water. It is improbable that the osmotic disturbances induced by the application or injection of water are the same as those effected by the application or injection of solutions. The fact that the various disturbances produce responses differing only in degree would seem to indicate that the causes determining the formation of intumescences have not yet been fully analyzed into their separate factors. It is not unlikely that different plants react differently in this respect. The experiments of Steiner would seem to indicate that such a possibility exists.—H. Hasselbring.

**Taxonomic notes.**—Gates\(^11\) has attacked the genus *Polygonatum*, which he says "has been in a very chaotic condition owing to the lumping of species, the transference of names, and the confusion of North American with European

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