tumor sectors of a single individual and the physiological comparison of these cell lines in tissue cultures. While this is theoretically possible in animal and human tumors, it is fraught with such technical difficulties that it has not yet been accomplished over more than very brief periods, whereas in these spruce tumors it can be done with relative ease.

We believe that these tumors represent a promising addition to our roster of materials in which the processes of tumefaction can be profitably studied.

1 P. R. White, Quart. Rev. Biol., 26, 1–16, 1951.

A PHYSIOLOGICAL BASIS FOR AUTONOMOUS GROWTH OF THE CROWN-GALL TUMOR CELL*

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We are commemorating on this occasion the description of a rather remarkable micro-organism. This bacterium, which is now known as Agrobacterium tumefaciens (Smith and Town.) Conn, is responsible for the initiation of the non-self-limiting neoplastic disease of plants known as crown gall. The isolation and characterization of a tumor-inducing bacterium shortly after the turn of the century attracted considerable interest among pathologists generally because at the time of that discovery no animal tumor had yet been produced experimentally. For a period of about twenty years Erwin F. Smith made detailed comparative studies of crown-gall and malignant animal tumors and found that these two types of growth had much in common. There appeared, however, to be one fundamental difference. Smith believed that the continued unregulated proliferation of the crown-gall tumor cell was dependent upon continued stimulation by the inciting bacterium. Crown gall was, therefore, not generally accepted by oncologists as being comparable to true animal tumors because, as described by Smith, this plant disease appeared to be simply a bacterial-stimulated hyperplasia and not a truly independent growth, as are most animal cancers.

In certain plant species such as the sunflower and Paris daisy there may be produced, in addition to a primary crown-gall tumor, secondary tumors that arise at points distant from the seat of the primary growth. These secondary tumors are interesting because they are frequently free of the bacteria that initiate the primary tumor. The finding that many of the secondary tumors are bacteria-free permitted the unequivocal demonstration of the truly independent nature of the crown-gall tumor cell.1 Sterile tissue isolated from the secondary tumors grew profusely and indefinitely on a culture medium that did not support the continued growth of normal cells of the type from which the tumor cells were derived. Small fragments of such tumor tissue implanted into a healthy host developed again into
typical crown-gall tumors. Since such sterile cells isolated not only from secondary tumors but subsequently also from primary tumors of many plant species have not, in the more than ten years that they have been kept under observation, shown the slightest tendency to become less autonomous, they have generally been regarded as being permanently altered cells that reproduce true to type and against the growth of which there is no control mechanism in the host. These are the characteristics by which malignant animal cells are distinguished from healthy or merely inflammatory cells. Thus the one basic difference thought by Smith to distinguish crown-gall from malignant animal tumors has been removed.

In any analysis of a complex series of events such as those that occur during tumor formation, it is often convenient to subdivide, in so far as that is possible, the total result into a series of contributing events, each of which is essential for the consummation of the completed process. In studying these events in the crown-gall disease, two distinct phases have now been recognized. In the first phase normal cells are altered to tumor cells which do not as yet develop into a neoplastic growth. Two known requirements must be satisfied to complete the inception phase. These have been termed "conditioning" and "induction." By "conditioning" is meant that only those plant cells that have been rendered susceptible as a result of irritation accompanying a wound can be altered to tumor cells. An excellent correlation has been found to exist, moreover, between the stage in the normal wound-healing cycle in which normal cells are converted to tumor cells and the size and rate of growth of the resulting tumors. Induction, on the other hand, refers to the actual conversion of a conditioned host cell into a crown-gall tumor cell by an as yet essentially uncharacterized tumor-inducing principle elaborated by the inciting bacteria. Induction completes the first phase of tumor formation. The second phase of tumor formation, according to this concept, is concerned with the continued unregulated and autonomous growth of the tumor cell, once the cellular alteration has been accomplished. The development of a satisfactory explanation for the continued abnormal growth of a tumor cell in the absence of any recognizable infective agent represents a very real challenge not only to students of crown gall but also to those interested in autonomous growth generally. Perhaps the most important single concept that has arisen from the plant work is that concerned with the nature of the autonomous growth of a tumor cell. It is with that aspect of the problem that we should like to concern ourselves at this time.

In order to gain insight into the nature of autonomous growth, it is necessary to understand something of the processes involved in normal growth and development. Growth in all higher animals and plants is the result either of an enlargement of the constituent cells of such organisms or of the combined processes of cell enlargement and cell division. These fundamental growth processes appear to be dependent for their development in plant cells upon specific substances that may be synthesized by plant cells themselves. It is now possible, moreover, to delimit under fully controlled experimental conditions, with the use as a test object of certain plant-cell types, these two growth processes. When, for example, tobacco pith parenchymal cells are treated with synthetic growth substances of the auxin type such as naphthalene acetic acid, the pith cells enlarge greatly in size but do not divide. It is only when a second growth factor such as 6-furfurylaminopurine, or the naturally occurring equivalent of that substance, is supplied to the pith
parenchymal cells in addition to an auxin that a profuse growth accompanied by cell division results. Without an auxin, 6-furfurylaminopurine is ineffective in encouraging either an enlargement or a division of the pith cells. These findings demonstrate that two growth substances, one of which is concerned with cell enlargement and the other with cell division, act synergistically to promote growth and cell division in tobacco pith parenchymal cells. Normal tobacco pith cells do not and cannot themselves synthesize these two growth substances, for, if they did, they would respond in the characteristic manner indicated above. Since the cellular systems responsible for the synthesis of these two growth substances appear to be solidly blocked in normal tobacco pith cells, it was of interest to learn how such cells would respond when transformed to crown-gall tumor cells. The results of these studies, which are reported in detail elsewhere, demonstrated that when healing pith cells are converted to crown-gall tumor cells, typical crown-gall tumors develop.\(^{11}\) This simple experiment demonstrates that, although normal tobacco pith cells did not and could not synthesize physiologically effective concentrations of either a cell-enlargement or a cell-division factor prior to their conversion to tumor cells, both substances were synthesized in greater than regulatory amounts following alteration. If this were not true, continued growth accompanied by cell division and hence tumor formation would not have resulted in the test system used in these experiments. It is clear, therefore, that an essential difference between a normal tobacco pith cell and a crown-gall tumor cell appears to be concerned at a physiological level with the permanent activation of two growth-substance-synthesizing systems, the products of which are concerned specifically with growth accompanied by cell division. The continued production in greater than regulatory amounts of the cell-enlargement and cell-division factors by the tumor cell could account for the continued abnormal proliferation of such a cell. Subsequent studies have shown,\(^{12,13}\) however, that additional metabolic systems are permanently activated during the transition from a normal cell to a fully altered, rapidly growing type of crown-gall tumor cell. It has been found that alteration of normal cells to tumor cells is a gradual but progressive process.\(^{14}\) When, for example, the tumor-inducing principle responsible for inception of the crown-gall tumor is allowed to act on plant cells for only 34-36 hours before being inactivated by thermal treatment, small, very slowly growing benign growths are elicited in a host. A 50-hour exposure of cells to the action of that principle results in tumors that grow at a moderately fast rate. If the tumor-inducing principle is allowed to act for 72-96 hours before being destroyed by heat, rapidly growing, potentially malignant tumors result. It is also possible to obtain tumors showing varying degrees of neoplastic change by allowing the tumor-inducing principle associated with slightly virulent or moderately virulent strains of the crown-gall bacteria to act on host cells throughout a 4- or 5-day period. Sterile tissue isolated from the three types of tumors described above and planted on White's basic culture medium retain indefinitely their characteristic growth patterns. This is illustrated in Figure 1. Since these three types of tumors were derived from the same plant species, they were admirably suited for a study of the factors required for rapid autonomous growth. In these studies, the results of which are summarized in Figure 1, the rapidly growing, fully altered tumor cell was used as the standard. This cell type can synthesize, in optimal or near-optimal amounts all the growth factors needed for its
continued rapid abnormal growth from mineral salts and sucrose present in White's basic culture medium. The moderately fast-growing tumor cell required that the basic medium be supplemented with glutamine, meso inositol, and a cell-enlargement factor (naphthalene acetic acid) to achieve a growth rate comparable to that of the fully altered, rapidly growing type of tumor cell. The very slowly growing benign tumor cells altered in a 34-hour period required, in addition to the three compounds described above, asparagine as well as cytidylic and guanylic acids to achieve full growth. Asparagine did not, however, represent an absolute require-

![Fig. 1—Relative rates of growth of three clones of crown-gall tumor tissue that show different degrees of neoplastic change, planted on White's basic medium. (Left), Fully altered rapidly growing tumor cells. (Upper left), Moderately fast-growing tumor cells. (Upper center), Very slowly growing tumor cells. (Upper right), Normal cells of the type from which the tumor cells were derived. While the three clones of the tumor cells grow continuously although at different rates on the basic culture medium, normal cells of this type do not grow on that medium. Lower pictures and legends show minimal nutritional supplements needed by the three types of tissues to achieve a growth rate comparable to that of the fully altered tumor cell. (Photographs by J. A. Carlile.)](image)

ment for rapid growth but served to stimulate somewhat the growth of tumor tissue fragments planted on an otherwise suitable culture medium. Certain amino acids, particularly proline and histidine, also appeared to have a stimulatory effect on the growth of this tissue, but this requirement seemed to be of a transient nature, since the amino acids did not appear to be required in the second transfer of the tissue to the supplemented medium. It is clear from these experiments that, as the crown-gall tumor cell becomes more autonomous, its requirements in terms of externally supplied growth factors become less exacting. More importantly, however, these
studies clearly demonstrate that a series of well-defined growth-substance-synthesizing systems become gradually activated during the transition from the normal cell to the fully altered tumor cell, and the degree of activation of these systems determines the rate of growth of the tumor cell.

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

The concept of growth autonomy presented above finds additional support in other directions. It has been possible to reproduce under precisely defined experimental conditions and with the use of certain normal cell types as a test object not only the morphological growth patterns\textsuperscript{10,12} (slow and rapid disorganized growths, teratoma-like structures) but also the histological (hypertrophy and hyperplasia leading to disorganization and loss of function) as well as the cytological
(multinucleate giant cells, etc.) abnormalities that characterize the tumorous state in crown gall. This was accomplished by varying the proportions of the cell-enlargement factor and the factor limiting for cell division in an otherwise suitable culture medium on which the normal cells were planted. These artificially stimulated normal cells, in contrast to crown-gall tumor cells, are self-limiting growths, and, when the externally supplied stimuli are removed, their growth promptly stops. The fact that such stimulated normal cells commonly show histological and cytological characteristics of true tumor cells but are themselves self-limiting growths indicates that the observed cellular abnormalities are the result rather than the cause of the tumorous state.

The results of all of these studies strongly suggest that it is possible for a cell to acquire the capacity for autonomous growth as a result of the permanent activation of a series of growth-substance-synthesizing systems, the products of which are concerned specifically with growth accompanied by cell division. These systems are precisely regulated in all normal plant cells.

* This investigation was supported in part by a research grant (PSH, C-2944 M & G) from the National Cancer Institute, Public Health Service. This paper was presented at the National Academy of Sciences, Symposium on Plant Tumors, autumn meetings, November 19, 1957.


3. A. C. Braun and T. Laskaris, these PROCEEDINGS, 28, 468, 1942.


