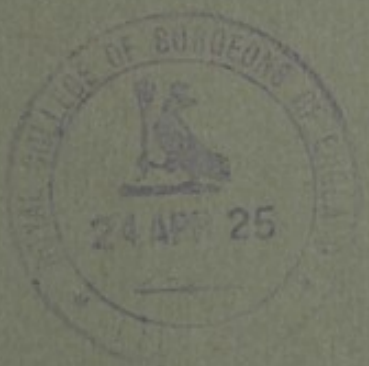
On some resemblances of crown-gall to human cancer / Erwin F. Smith.

ON SOME RESEMBLANCES OF CROWN-GALL TO HUMAN CANCER

ERWIN F. SMITH



[*Reprinted from* SCIENCE, *N. S., Vol. XXXV., No. 892, Pages 161–172, February 2, 1912*]

[*Reprinted from* SCIENCE, *N. S., Vol. XXXV., No. 892, Pages 161–172, February 2, 1912*]

ON SOME RESEMBLANCES OF CROWN-GALL TO HUMAN CANCER[1]

THE disease on which I shall speak to-day is known in this country as crown-gall, because it has been observed most frequently on the crowns of trees and shrubs, but it is not peculiar to this situation. It occurs also on roots and shoots. This disease has been known to cultivators and to plant pathologists for many years and has caused more or less injury to a variety of plants both in this country and in Europe. Of plants subject to serious injury may be mentioned: Roses, almonds, peaches, raspberries, grapes. Sometimes the plants are only dwarfed or crippled, at other times killed. Recovery, especially in certain species, is frequent. In Italy the attacked grape vines are said to live about four years.

It has been ascribed to a variety of causes, *e. g.*, frosts, wounds made in cultivating, insect injuries, fungous injuries, physiological disturbances, etc. The actual cause was not known until discovered by the writer and his associates. Team work on this disease has been carried on in the U. S. Department of Agriculture for the last eight years, *i. e.*, since February, 1904. The first successful pure culture inoculations were obtained in 1906. The organism was described and named by us in 1907.[2]

[1] Address as retiring president of the Botanical Society of America, Washington, D. C., December 28, 1911. By invitation members of the following organizations were also present: Section G, of the American Association for the Advancement of Science; Society of American Bacteriologists, and the American Phytopathological Society.

[2] SCIENCE, N. S., Vol. XXV., No. 643, pp. 671–673, 1907; see also *Centralb. f. Bakt.*, 2 Abt., XX. Bd.

Addresses setting forth the parasitic nature of the organism have been given before this Society by Dr. Townsend, and before the Society of American Bacteriologists and the American Phytopathological Society by myself.[3] I have also twice in public addresses before the American Association for Cancer Research called attention to certain general resemblances of this disease to malignant human tumors, namely, at the Boston meeting in December, 1909 (lantern-slide address), and again in the spring of 1910 at the Washington meeting of the Association, where I showed specimens of the disease. The whole subject so far as regards the etiology of the disease was also summed up in a big bulletin published by the Bureau of Plant Industry, U. S. Department of Agriculture, early in 1911.[4] I may assume, therefore, that this audience is fairly well acquainted with the evidence adduced by us to prove the pathogenic nature of the organism we have called *Bacterium tumefaciens*, and therefore I shall not spend any time on this phase of the subject. Those who are not familiar with the evidence can easily obtain the necessary publications and if these are not convincing they may repeat the experiments.

In a brief way I have also published on the newer discoveries upon which I am to speak to-day, *i. e.*, in a third address be-

[3] Vide SCIENCE, February 12, 1909, p. 273; *ibid.*, August 13, 1909, p. 223; and *Phytopathology*, 1911, Vol. I., p. 7.

[4] No. 213, "Crown-Gall of Plants: Its Cause and Remedy." To be had from the Superintendent of Documents, Government Printing Office, Washington, D. C. Price 40 cents.

fore the American Association for Cancer Research,[5] an abstract of which was published by the Department of Agriculture as Circular No. 85, Bureau of Plant Industry, and in *Zeitschrift f. Krebsforschung*, 11 Bd., 1 Heft. Since that date the subject has been studied continuously. Numerous sections have been prepared, and I will show you lantern slides of photomicrographs made from some of these sections, so that you will be able to judge for yourselves as to the bearing of the evidence.

It is hardly possible to say who first noted the superficial resemblance of overgrowths on plants to animal tumors. It probably goes far back of published records, since we have in English the word "canker" applied to certain of these overgrowths, which word is only another form of the word cancer. Also in German, the word "Krebs" is applied indifferently to these overgrowths and to malignant human tumors. It is one thing, however, to find a superficial resemblance of plant diseases to animal diseases, and quite another to establish any strict analogy. In fact, as histological studies on cancer have multiplied animal pathologists have been more and more convinced that there is no real likeness between the plant overgrowths and malignant animal tumors, and this is true enough, I believe, for club-root of cabbage, the plant disease most studied in this connection. A comparatively recent statement by Alfred Fischer that the only thing they have in common is the name (Krebs) may be taken as fairly representing the current view.[6] I shall hope, however, to show you before I am through that they have a good deal in common, so much, in fact, that I believe we have in these particular plant overgrowths a key to unlock the whole cancer situation. In consideration of these discoveries many closed doors in cancer research must now be opened and studies on the etiology of the disease must be done over with a view to finding a parasite within the cancer cell, and separating it therefrom by an improved technic of isolation. Before I show you any slides or describe further the discoveries made it will be necessary for me to refer briefly to the nature of cancer and certain other malignant animal diseases.

When I first called attention of members of the American Association for Cancer Research to crown-gall in 1909, the reply of some of the members was that while I had demonstrated crown-gall to be a very interesting disease it was evidently a granuloma, and not a true tumor. With this conclusion I can not agree. That you may understand why crown-galls are not granulomata I wish briefly to call your attention to the phenomena occurring in such diseases. As example of a granuloma, we may take tuberculosis. We have in this disease a focus of infection and source of irritation in the presence of a microorganism. Against this organism the body reacts with the formation in the immediately surrounding tissues of cell growths not unlike those which occur in the bottom and sides of wounds, namely, granulation tissue, hence the name granuloma. In this manner nodular growths arise, but these nodular growths are limited in extent of tissue involved, are produced from the tissues immediately surrounding the bacterial nest, are not vascularized, and soon become disorganized in their interior. In tuberculosis the blood vessels occurring naturally within the attacked area are obliterated and excluded from the tubercle; in certain other granulomata, *e. g.*, syphilitic gummata, the vessels are not obliterated, but they are distinct in other ways, *e. g.*, enclosed in a fibrous capsule. The disease

[5] Buffalo, April 13, 1911.

[6] *Vorlesungen ueber Bacterien*, 2te Auflage, 1903, p. 277.

is carried from place to place within the body by the migration of the microorganisms, either in the blood stream or the lymphatics or in some other way, *e. g.*, through the digestive tract. Wherever these migratory organisms lodge they set up or may set up similar irritations with the production of similar nodules of granular tissue, the same being an effort on the part of the infected animal to overcome the disease. The point which I wish specially to emphasize is the fact that in these secondary infections the granular tissue which develops is formed out of the particular organ in which the parasites happen to lodge, and does not consist of cells brought to it from a distance.

In this respect cancers are quite different. Parenthetically I might stop here long enough to say that I shall for the purposes of this address use the term cancer in a loose, general sense for all malignant human tumors. First, because the crown-gall which I have studied seems to partake of the nature of different types of malignant animal tumors, and because I believe that when the cause of malignant animal tumors is discovered we shall find that many of the hard and fast lines of separation which the animal histologists have erected between sarcoma, carcinoma, etc., will be found untenable.

In cancer we have an enormous multiplication of certain tissues of the animal (epithelial, connective, etc.) which by continued growth crush and disorganize the surrounding tissues. These growths are more or less highly vascularized, and new vessels are formed as the tumor develops, but not to an extent sufficient to carry on the growth beyond a certain point. Usually there is a great excess of parenchyma cells in such a tumor and the blood vessels are not sufficiently numerous to nourish it properly, so that after a longer or shorter period (months or years) portions of it disorganize often into open wounds which are then readily infected by all sorts of secondary organisms with all the well-known disastrous results. This then is one striking difference between granulomata and cancers, but the true nature of the cancerous development becomes more evident in the secondary tumors. The mere fact that a primary cancer has developed on some part of the body does not constitute the chief danger, since one might have such a tumor for a long time without death supervening, unless the primary growth happened to be situated in or near a vital organ. What constitutes the peculiar malignancy of cancer is the tendency to form secondary growths in various parts of the body, including the vital organs, and it is this clearly recognized danger which in modern times has led to the universal recommendation on the part of competent physicians and surgeons of the early extirpation of suspicious growths, the hope being that the surgeon may be able to dissect out all the infected tissues and thus free the patient from the disease. This is the reason why, for instance, in cancer of the breast the surgeon so carefully removes not only the infected breast, but the lymphatics for long distances away, that he may, if possible, reach beyond the unseen growing cancer strands. This also is why delayed operations for cancer are seldom successful.

In case of granulomata, as we have seen, it is the parasite which migrates. In case of cancers it is the cancer cell itself which migrates, *i. e.*, some of the body cells which under some unknown stimulation have been taken out of the physiological control of the body and have become thus, as it were, parasites on their fellow cells. There are two ways in which secondary tumors are derived from the primary tumor in cancer: (1) The primary tumor growing peripherally sends out roots or

strands which bore their way through normal tissues of the body, sometimes for long distances, developing from certain portions of these strands secondary tumors. (2) Small groups of cancer cells are dislodged from the parent tumor and carried as floating islands in the blood stream or lymphatics to develop secondary tumors where they lodge. The first of these ways has been definitely established by observation; the second by inference, no connecting strand having been discovered. Naturally these secondary tumors, being derived from the primary tumor, tend to partake of the nature of the tissue from which the primary tumor has developed. For example, if the primary tumor be in the stomach, the secondary tumors are likely to contain glandular cells resembling those of the stomach, wherever they may be developed. This is such a striking peculiarity that it is often possible for the animal pathologist to tell from the study of his sections whether the cancer is primary or secondary, and, if secondary, in what organ the primary tumor is located. In case of tumors located in an organ containing all three of the embryonic layers or developed out of cell-rests of this nature we might have in the tumors a jumbled-up mass of all sorts of tissues—skin, bone, teeth, hair, muscle, nerve, etc. This at least is one method of explaining the embryomata.

Having found no parasite in the cancer cells, a majority of the animal pathologists have given up the idea that cancer can be of parasitic origin. For a generation the research workers fell back upon Cohnheim's hypothesis that cancers were due to the development of small fragments of tissue cut off from the parent layer during embryonal growth, to be enclosed in other tissues and lie dormant until acted on abnormally later in life by some unknown stimulus. But while studies of the animal body show that such separation of small portions of tissue from the germinal layer is not uncommon, research workers on cancer are now generally agreed, I believe, that there are many phenomena connected with the development of cancer for which this hypothesis of Cohnheim offers a wholly inadequate explanation. Moreover, what induces these dormant cells to develop was never determined. A very favorite theory with cancer specialists has been that the cancer cell itself is the only parasite, and that no infections could be obtained on animals unless the living cancer cell were present. This hypothesis must now be abandoned owing to the discovery by Peyton Rous (1911) that sarcoma of chickens may be produced in the absence of cancer cells, *i. e.*, by cancerous fluid filtered free from all traces of living cancer cells. So far as I know he has not expressed any opinion as to the nature of the infection which has been separated from his ground chicken sarcomata by centrifuging and also by filtration through Berkefeld bougies, but in the light of the evidence we have secured from plants I believe you will agree with me that it can be nothing else than a living microorganism, minute enough to pass through the walls of the rather coarse filter.

In crown-galls I have not found the second method of formation of secondary tumors, namely, by the detachment of small fragments of the primary tumor to be carried in a stream and lodged at a distance. This method we should hardly expect to find in plants, owing to the fact that there is no rapid blood stream such as we find in animals, neither does it seem to be more than an epiphenomenon in tumor growth, the essential thing being the abnormal internal stimulus to cell division. The first method of propagation, namely, by strands, occurs, however, and parallels to my mind very strictly what occurs in

malignant animal tumors, *e. g.*, in carcinoma, sarcoma, etc.

The existence of the tumor strand in crown-gall was overlooked for a long time. But last spring in making some sections of Paris daisy plants which had been inoculated with the crown-gall organism and bore both primary and secondary galls, I saw on cross-section a tumor-strand in the inner wood next to the pith between the secondary and the primary tumor and near the latter. This was about a millimeter in diameter and of a different color, *i. e.*, greenish, and easily observed by any one. Often, however, this strand is composed of a few cells only and difficult to find, even with the compound microscope. This, together with preoccupation on other phases of the research, must serve to explain why it was overlooked for so long a time. As soon as I saw this parenchyma out of place I said, "Here is a tumor strand!" and began to examine many other plants to see if it was at all constant—finding it visible to the naked eye near the primary tumor in perhaps 20 per cent. of the plants examined. The question then arose whether it was merely local, or could be traced for some distance and was of constant occurrence in the normal tissue between the primary and the secondary tumors. Since then many inoculated plants have been examined microscopically, and in all of them I have been able to find this tumor strand, although, as already stated, in many cases it is composed of a very few cells. In the Paris daisy it usually bores its way between pith and wood, or at the inner edge of the wood wedge in the protoxylem, apparently along lines of least resistance. (Lantern slides were exhibited, showing cross and longitudinal sections of such strands from the inoculated plants.)

On this strand are developed secondary tumors, apparently either where the food supply is most abundant or where the pressure of surrounding tissues is least, yet possibly other factors are involved. Frequently the growth of the strand is rapid. In very juicy favorable material in 16 days from the date of the primary inoculation I have seen secondary tumors develop from such strands at a distance of 10 centimeters from the parent tumor. Often deep in the resistant wood the tumor strand is under great pressure. In softer parts the overlying tissues are split open, and the deep secondary tumor then comes to the surface. In the Paris daisy, when the primary tumor is on the stem, secondary tumors often develop on the leaves, and strands of tumor tissue have been traced in numerous instances all the way from the primary tumors through the stem into the leaf, and all stages of the development of the secondary tumors observed on many plants. This tumor strand boring its way through stems and leaves appears to be as much a foreign body as the roots of a mistletoe or the mycelium of a fungus. From these strands and from these secondary tumors we have isolated the same microorganism that occurs in the primary tumors and with subcultures from such bacterial colonies have reproduced the disease. The discovery of this strand affords a satisfactory explanation for the fact that the morbid growth usually returns after excision.

The second striking fact to which I wish to call your attention is that when the primary tumor occurs in the stem and the secondary tumor in the leaf the structure of the secondary tumor is not that of the leaf in which it is growing, but of the stem from which the strand was derived. If the discovery of the strand was an accident, this latter discovery was reasoned out, knowing what takes place in cancer. I said immediately, if this is a tumor-strand we ought to find a stem-structure in the leaf tumors, and the very first leaf tumors

cut showed typical examples of it. In secondary tumors occurring in the leaves as the result of stem inoculations the development of a stem consisting of a loose, rapidly growing parenchyma in the center, surrounded by wood wedges separated by medullary rays, beyond which is a cambium zone and a bark can be made out very clearly (slides exhibited). Sometimes these secondary tumors develop a very perfect stem structure; often, however, the stem is more or less imperfect with the inclusion of large parenchyma cells of the leaf, and with a great overproduction of stem parenchyma (medullary rays, etc.) as compared with the vascular portion. As this secondary tumor grows the surrounding leaf structure is destroyed, and eventually we may have a growth which bears no resemblance whatever to a leaf. Often, however, fragments of the leaf adhere to the surface of the tumor, and show an unchanged leaf structure.

These secondary leaf-tumors then, so far at least as regards the parenchymatous portion, are composed, in great part at least, of descendants of the originally infected stem-cells. The growth is an invasion of infected cells. To what extent neighboring uninfected cells are also involved is uncertain. The wood always shows hyperplasia, sometimes to a very marked degree in the vicinity of a stem tumor, and usually also in the vicinity of the tumor strand, especially if this is large. Are all of these wood cells infected? Probably not. I see no reason why we might not have changes in the plant distantly comparable to the inflammatory changes which take place in the vicinity of a malignant animal tumor, *i. e.*, an excessive multiplication of cells which while a part of the tumor are not its malignant portion. This must be left for further study.

This astonishing stem structure in leaves is quite parallel to that which occurs in certain cancers of secondary origin where the structure of the primary tumor is outlined, albeit often only imperfectly. We might now inquire whether primary tumors produced on leaves do not have the same structure as those just described as secondary tumors. We have made needle-puncture inoculations on leaves of Paris daisy and have studied the structure of the tumors which develop and these do not have a stem structure but an irregular epithelioma-like structure derived wholly from the leaf, as may be seen from the lantern slide exhibited.

What happens finally in the case of cancer happens in crown-gall, namely, the tissues not being sufficiently vascularized, and composed of a great excess of soft and fleshy cells, are easily disorganized with the production of open wounds. In case of crown-galls on the daisy and many other fleshy plants after about two or three months large portions of the tumorous tissue decay with the formation of open wounds, subject to a variety of secondary infections.

It should be stated here, however, that in the crown-gall there are no abscess cavities such as we find often in granulomata or in such a disease as olive tuberculosis. Sometimes there is a multiplication of bacteria in the vessels in the vicinity of the needle puncture, but whether these are the crown-gall organisms or not we have not yet determined. Certain it is that when the tumor has begun to grow rapidly no bacteria or other granular matters have been found in the vessels or in the intercellular spaces. The causal bacteria occur inside the cells, which are stimulated by their presence to multiply with great rapidity and without reference to the physiological needs of the plants, *i. e.*, the plant has no direct control over the growth.

In these particulars crown-gall resembles epitheliomatous growths, while in the em-

bryonal character of its luxuriant granulations and in its predilection for young plants and rapidly growing tissues it is more like sarcoma. The growth is a hyperplasia rather than a hypertrophy, although occasional groups of large cells occur. There can be no doubt as to the development of new vessels in the growing tumor. This is shown clearly by the anatomy of the secondary tumors. Whether the vessels are ingrowths from the surrounding tissues, or outgrowths from the tumor strand, or both, as would seem to be the case, must be left for further inquiry. The anatomy is unlike that of club-root of cabbage, where the growth consists of an enormous enlargement of a comparatively few infected cells.

I think, therefore, that we have in crown-galls a striking analogy to what occurs in malignant animal tumors, namely, to recapitulate, *the cell itself a disturbing force, i. e.,* an enormous multiplication of certain cells of the body without reference to physiological needs and in opposition to the best interests of the organism; a non-capsulate tumor, with absence of abscess cavities and of plainly visible parasites; peripheral growth and a well-developed stroma consisting of vessels and fibers; from this primary tumor the development of strands of tumor tissue upon which secondary tumors develop; in the secondary tumors a strong tendency to take on the structure of the organ in which the primary tumor has developed; frequent if not necessary origin of the primary tumor in bruises, wounds or irritated places; complete recovery if all the tumor tissue is extirpated, failure if it is not; in some cases spontaneous recovery. The chief difference so far made out is that in case of cancer cells we know nothing whatever as to the cause of the abnormal growth,[7] whereas in case of these overgrowths on plants we have definitely proved them to be due to the presence of an intracellular schizomycete which we have many times isolated and reisolated in pure culture and by means of which we can reproduce the disease at will.

The question now arises whether animal tumors might not be produced by means of the crown-gall organism. I might state here that while I believe cancer to be due to some intracellular microorganism which in its physiological peculiarities, action on the cell nucleus, etc., is like the one we have discovered, I do not maintain the overgrowths in warm-blooded animals to be due to this particular organism, for the reason that its maximum temperature for growth (daisy strain) is a little under the blood temperature of such animals. In thinking over the matter it seemed to me not unlikely, however, that with this organism I might be able to produce tumors in cold-blooded animals, and so four years ago I attempted to do it. I will show you only a slide or two made from an inoculated fish. I used for this purpose brook trout and in a very considerable portion of my inoculations I succeeded in producing ulcers in the deeper tissues where the needle entered. In this instance the needle entered the belly wall of the fish. The wound healed externally, but at the end of 21 days when the trout was dissected there was a well defined inner growth (proliferation nodule) in the connective tissue between the muscles with formation of giant cells. There were also when dissected two external sore spots, one below the pectoral fin and the other below the anal fin, both of recent occurrence, but no throat or gill ulcers in this

[7] "Some unknown force, the essential nature of which has so far completely escaped our knowledge and our comprehension, is capable of calling forth this latent power of proliferation, and the germ [cancer cell] begins to grow *out of itself*, like a seed that has been buried in the ground." (Dürck.)

fish. Similar growths were obtained in the eye-socket. I showed sections cut from one of these ulcers to one of the most distinguished research workers on cancer in this country and he said "if we had this in man we should call it sarcoma." (Slides exhibited.) I propose to repeat and extend the work on trout and therefore will say but little about this phase of the investigation.

In conclusion I wish to call attention to some of the peculiarities of the microorganism (*Bacterium tumefaciens*) as determined by our cultural work. As well known to many of you, we prosecuted our studies upon the crown-gall for two years before we were able to isolate the parasitic organism. Ten years previous to this I spent six months on the subject with a similar negative result. Two obstacles of which we were unaware blocked the way. In the first place the organism in a viable form occurs in the tumor tissue of the daisy in small numbers only. If inoculations are made from crown-gall tissue, using about that amount of tissue we are accustomed to use for other bacterial plant diseases, and also for many animal diseases, the chances are that no colonies of the parasite will be obtained upon the plates. I have no doubt now that we made dozens of plates—yes, I might say dozens of separate sets of poured plate cultures, on which not a single colony of the right sort developed. It was only when we learned to inoculate our bouillons and agar plates with large quantities of the tumor material that we were able to obtain a sprinkling of colonies of the right organism. From a young, rapidly growing tumor it is always possible to obtain the organism with proper technic and sometimes in pure cultures, but often only by using a hundred, a thousand, or a hundred thousand times too much material, if one were working with other organisms. The second obstacle is the fact that the living bacteria in the tumor tissue occur for the most part in a paralyzed condition, either as involution forms or in some other form which does not grow readily when plates are made. Cultures were made every few weeks from crown-gall tissue for two years and numerous and various bacteria were obtained on these plates, pricked off for sub-culture, studied microscopically and culturally, and inoculated into the plant with negative results, these organisms being the saprophytes which usually accompany crown-gall. The plates were usually discarded after three or four days, and so the work went on. If, however, one inoculates copiously as described, and waits a week or ten days for the paralyzed organisms to recover their vigor, he will then obtain colonies of the parasite.

Two questions arise: (1) Why does an organism which produces such striking results occur in the tissue in such small numbers? (2) What paralyzes it so that when agar plates are made from the tissues the colonies do not appear until the fourth, fifth, sixth, eighth or tenth day, and sometimes not until the twentieth day? These questions have received a good deal of thought. After a time we discovered that when the organism is grown in bouillon or other media containing sugar an acid is produced, and it then occurred to me that this acid might be the cause of the death of a large proportion of the organisms in the cells, and of the paralyzing of the remainder. Peptone water flask cultures of the organism were then grown in the presence of sugar and turned over to the chemist, who reported that the acid present was acetic acid. We found that after a time all the organisms in such cultures were dead, and a microscopic examination showed that a large proportion of them occurred in the form of irregular club-shaped or Y-shaped bodies, *i. e.*, they had passed over into involution forms preced-

ing their death. Subsequently we found that on adding dilute acetic acid to fresh cultures of the organism either on agar or in bouillon we could at will produce these involution forms. Ordinarily it was found on making poured plates from such cultures that all the organisms were dead, but by further experimenting we learned that if we added just the right quantity of acid, involution forms were produced and a portion of the bacteria killed, but that some remained alive and those which remained alive were paralyzed, coming up on the agar plates in the same slow manner as those from the interior of the tumors. I should have stated that although the organism comes up slowly from the crown-gall on agar-poured plates, subcultures from such colonies grow as readily as from any other easily cultivable organism, *B. coli,* for example, showing clearly that the initial slow growth is not a peculiarity due to differences in culture-media or inherent in the organism, but only one due to its previous environment in the plant cell.

Do the same phenomena occur in the plant cell? Recently from crown-gall of daisy grown for the purpose, the chemist has isolated for us an acid which he says is acetic acid. We have also found in the tissues numerous bacterial Y-shaped bodies, such as occur in our flasks when acetic acid is present. I think, therefore, we may assume, tentatively, at least, that an acid in small quantities is formed also in the cells of the crown-gall as a by-product of the bacterial growth, and that after a time this acid stops the growth of the multiplying bacteria within the cells exactly as it does in the flask cultures, causing them to take on involution forms and killing the majority.

There is, as I conceive, a very delicate balance between the parasitic bacterium present in the plant and the activities of the plant cells. The cells of the plant are not destroyed by it, but only stimulated into rapid and repeated division. Upon its entrance into a cell, which must usually be by wounds, in our own experiments by needle-pricks, we may conceive the micro-organisms to multiply rapidly for a short time. The acid developed by this multiplication then inhibits the further growth of the bacteria, causing the appearance of Y-shaped bodies and the death of a certain proportion of the bacteria, sometimes nearly or quite all of them. The membrane of the bacterial cells which are killed is now permeable, and the bacterial endo-toxines diffuse out into the cell. The nucleus of the cell now immediately divides, under the stimulus either of the acid or of the aforesaid endo-toxines, or possibly from an excess of carbon dioxide due to the bacterial growth. There can be no doubt, I think, that carbon dioxide exists in excess in these cells, because the crown-gall tissues contain an excess of chloroplasts in the absence of any other visible means of obtaining this necessary food. These chlorophyll bodies are so abundant as often to give a distinct green color to deep tissues wherein we would ordinarily expect to find but few chloroplasts.

The next difficulty is to explain why the paralyzed bacteria carried over into the daughter cells suddenly begin a new growth. This can result, I think, only from the pouring out into the cell at the time of division of a fluid which was not previously present in it, namely, the nuclear sap which must flood the cell as soon as the nuclear membrane disappears. Whatever the explanation may be, the bacteria take on a new growth for a short time in the daughter cells with the reproduction of the already outlined phenomena. In this way occurs within a few weeks or months an enormous overgrowth

of the tumor tissue with the development of strands and of secondary tumors as already described. Using rapidly growing favorable plants, it is possible by means of a few needle-pricks carrying in the parasitic organism to obtain a tumor as large as one's fist in as short a period as six weeks. Ordinarily, however, growth is slower. Dr. A. P. Matthews, to whom I am indebted for suggestions respecting the effect of the nuclear sap on animal cells, tells me he has observed in case of the entrance of sperm cells into the eggs of star fish that the sperm retained its original form until the breaking up of the nuclear wall and the diffusion of the nuclear sap into the egg cell, whereupon the sperm took on a rapid growth.

Although we are able by means of poured-plate cultures to isolate the organism in a pure state from young crown-galls and reproduce the disease at will, we can not readily demonstrate the presence of the organism in the tissues by means of the microscope. If the bacteria were as readily seen in crown-gall tissues as they are, for instance, in the tuberculosis of the olive, the cause of the disease would have been discovered long ago. The organism is not an acid-fast organism, and when it stains at all a great variety of cell inclusions also stain and some of these derived from the cell protoplasm or from special parts of the nucleus are confusing. Its staining is also complicated by the fact of its passing over so readily into involution forms which are proverbially difficult to stain. I have seen occasionally inside of the cells of the crown-gall motile, flexuous, rod-shaped bodies which I take to be this organism, and we have occasionally stained in small numbers in the cells bodies which closely resemble rod-shaped bacteria, but ordinarily they occur in such small numbers or take stains so vaguely and imperfectly that this method of demonstration would not be convincing to an outsider. Also sometimes we find small groups of cells filled with what appear to be semi-disorganized bacteria, as if here the bacteria had gained the mastery for a short time and then degenerated. We have not in the whole eight years obtained any very satisfactory slides, although many attempts have been made, using a great variety of fixing agents and of stains. As I have stated elsewhere, if we had depended on the microscope alone we should not have been able to work out the etiology of this disease, and the plain demonstration of the parasite in the cells must await, I think, the development of some special technic of staining whereby we may be able to mordant the bacteria in such a way that they shall take one color while the contents of the host cell takes another. Even in case of the Y-shaped bodies one is seldom able to demonstrate them in the stained cells. We have obtained the best results by an indirect method, namely, by taking clean slides and burning the surface free from all possible organisms, then putting on a little distilled sterile water, and putting into this sections of young crown-galls taken from a portion of the tissue pared free from all exterior parts, allowing the contents of the cut cells to diffuse into the water for an hour, then removing the sections, drying the fluid and staining the slide. Examining such slides under the oil-immersion objective in course of a day one finds a good many such Y-shaped bodies. We have found the best method to be the systematic search of the whole slide, passing it back and forth under the objective. Searched in this way, about one field in four yields a Y-shaped body. Bacterial rods have also been obtained from the tissues in this way.

Various researchers on cancer have mentioned finding rod-shaped and Y-shaped bodies in cancer cells. For example, Dr.

Borrel, of the Pasteur Institute in Paris, and Dr. Reese, working in the cancer laboratory at Buffalo.

These plant neoplasms contain both small-celled and large-celled parenchyma and a variety of other tissues, *e. g.*, vessels and fibers. Cell division is sometimes so rapid that the cell wall can not keep pace. (Slides shown.) Frequently two and sometimes more nuclei are present in a cell. A portion at least of the cell divisions are by mitosis; but not all, it would seem. Some queer things take place in the cells. We are now studying the mechanism of cell-division in these tumors and are not ready to report.

To conclude, suppose we had in human cancer as its cause a microorganism multiplying in small numbers within the cell, having a definite action on cell nuclei, readily inhibited by its own by-products, losing virulence easily, passing quickly over into involution forms which are difficult to stain, and which are so paralyzed that only a very small portion will grow at all, except from the very youngest cells, and these only after a considerable period of time has elapsed, and further suppose that for their growth some very special technic of isolation, or some peculiar kind of culture media were necessary, then we should have precisely the same difficult conditions of isolation and determination as have confronted us in case of this similar overgrowth of plants, and ample explanation of why expert animal pathologists have been unable to see the parasite in their sections, and unable to cultivate it on their culture media, and consequently, have very generally reached the conclusion that it does not exist. Granted the existence of such an organism, and we have a ready explanation for the growth of the cancer cell in defiance of the physiological needs of the organism. The hitherto inexplicable occasional change in the nature of the cell-growth of tumors, *e. g.*, from epithelial to carcinomatous and from carcinomatous to sarcomatous also finds its explanation in the presence of a sensitive microorganism growing usually in the kind of cell originally infected but capable under certain circumstances of invading other types of cells.

ERWIN F. SMITH
U. S. DEPARTMENT OF AGRICULTURE

[The illustrations accompanying this address will be reproduced at an early date in a bulletin to be published by the U. S. Department of Agriculture.]