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A DESCRIPTION OF TUMORS ON IPOMOEA TUBA FROM THE A-BOMB TEST SITES ON ENIWETOK ATOLL

Appendix to Radiobiological Survey of Bikini, Eniwetok, and Likiep Atolls—July – August 1949

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Tumors on plants of Ipomoea tuba were found on Engebi Island during the radiobiological survey of July and August, 1949. At this time (17 months from the test shot on Engebi Island) many of the Ipomoea tuba plants located in an area 400 to 600 yards from the bomb crater showed tumorous growths of various sizes. The tumorous plants were found in disjunct areas of grass which had not been fully covered by the dense growth of Ipomoea which surrounded them (Figure 1).

This species of Ipomoea is a vine with large heart-shaped leaves and a stem which grows prostrate on the ground to a length of some ten meters (Figure 2).

The tumorous deformations on the plant varied from small warty out-growths at the nodes on the basal portions of the stem (Figure 3) to huge, convoluted tumorous masses completely covering a stem which had been reduced to only a few centimeters in height. The ability of the plants to recover from the deformation was indicated by the fact that the tumors were confined to the basal nodes in the first case mentioned above, and that even in the most severe cases normal leaves were occasionally produced from tumorous masses (Figure 4).

Morphological and physiological abnormalities were found in other plants and on some of the other islands surveyed. These were noted in the original report by both Biddulph and St. John (30) and included twisted stems and leaves, reduced leaves, abnormal fruits, double flowers, color changes, etc. So far as observed, Ipomoea tuba was the only plant to show tumorous growths.

Time and facilities did not permit a study of the tumors during the survey, but dried and preserved materials were brought back for study.

Since the problem of what causes abnormal plant growth and how it is maintained is one of the most fundamental in biology, we have made a survey of the literature on plant galls, or tumors, in an attempt to compare the tumors described in this study with some of those already known.

Plant galls or tumors may be caused by fungi, bacteria, viruses, nematodes, insects, chemical substances and genetic factors. They are as varied and numerous as the number of inciting agents would indicate.

The most intensely studied plant gall is known as Crown-Gall, which is incited by Agrobacterium tumefaciens. It has been observed on plants belonging to widely separated botanical families and it has been described on almost all organs of susceptible plants (10). The family Convolvulaceae, of which the genus Ipomoea, is a member is not found on this list, however. Crown gall bacteria are widely distributed and are apparently native in many soils where they lead an independent life or persist in old galls (16). It is uncertain whether the bacteria are intercellular or intracellular, but the bacteria must be introduced through a wound; and the size of the wound determines to some extent the size of the gall (9). Apparently the bacteria produce something which transforms normal tissue into tumor tissue. After this, the galls can continue to grow without the inciting principle, but the nature of the inciting principle and its mode of action are unknown. Crown-gall is not a systemic disease, however, and the relative size of the tumor apparently depends on the amount of transforming principle available at the time of the cellular alteration. There is a considerable histological variation in reaction to the crown-gall organism reported in the literature. In general, the tissues are more distorted than normal, and giant cells with many nuclei may be present (9). It has been reported by Braun (4) that cells which have undergone the transformation induced by the crown-gall organism can change back into normal cells and give rise to organs.

The host ranges of other gall-inducing bacteria are quite limited and are not applicable to this study (27).

Insects are probably the most common cause of galls in plants. In the case of a great many insects there is no mechanical injury, but in all cases there is a stimulatory effect on cells in a meristematic or plastic condition (11). As a result, there is cell enlargement or cell division or both, and the affected parts fail to differentiate into the characteristic tissues of the normal plant organ on which the gall is formed. The view is generally held that chemical secretions of the larvae are primarily responsible for the proliferations (6) although Rahn (26) makes a novel suggestion that radiation from the larvae may have some effect. There is a close analogy between the insect gall and the development of adventitious buds (7). On red currants, one of the mites produces a somewhat swollen bud, or a dense growth of buds which do not develop normally (1). Insect galls are not systemic, although evidence of systemic disturbance due to a multiplicity of insect bites has been presented in one case (25). Even in this instance, the effect did not extend for more than one or two internodes.

A number of workers have reported tumors on plants following the application of indoleacetic acid (5, 8, 31). When the histology of such tumors was studied by Kraus, Brown and Hamner (21) it was found that the cells of the endodermis were especially responsive. Root histogens developed and later gave rise to adventitious roots. Over the vascular bundles long proliferating strands of vascular tissues developed from endodermal derivatives. These frequently enlarged sufficiently to rupture the tissues exterior to them. Cambium, ray parenchyma, and xylem proliferated greatly. The responses reported for other plants have been much the same. Some investigators claim that the galls produced by indoleacetic acid and other organic acids are similar to those brought about by actual infection with the crown-gall organism (5, 22, 23). In all cases of tumors and overgrowths produced by growth substances, there is a marked proliferation of tissues which have already differentiated. The reports of adventitious shoots in cabbage (12), in Nicotiana hybrids (14), and in Geranium (30) indicate that somewhat normal recovery from growth substances is possible. The histological responses induced by 2, 4-D and 2, 4, 5-T are very similar to those of the other growth substances, except that they are more marked in degree. In some cases, there is stimulated cell proliferation and lateral root production, while in other cases, there is greater cambial activity and the formation of thick-walled xylem cells (29).

Viruses in plants usually do not affect the meristematic regions. "The continued normal functioning of the meristem of the growing points and cambium regions of most virus-affected plants indicates that if viruses are present in the meristem they rarely cause appreciable direct injury to this type of tissue" (2). An exception to this statement is the virus-produced tumor reported by Kelly and Black (20). This tumor arose chiefly in the pericyclic region of roots and stems and consisted of groups of distorted tracheids surrounded by meristematic cells and parenchyma interspersed with phloem.

Description of Tumors on *Ipomoea tuba*. -

The tumors varied from small wart-like tubercles at the nodes to large contorted masses 5-6 centimeters in circumference (Figures 5_a and 5_b). The tumors were yellowish-green in color, but the leaves produced on the newer growth above were normal in color. As has already been stated, leaves sometimes arose from the tumorous masses themselves, indicating at least partial recovery.

Histology. -

Whole tumors were brought to the laboratory in FAA. Pieces of the tumor tissue were dehydrated and cleared in an alcohol-chloroform series and inbedded in "Tissuemat." They were sectioned serially at 15u and stained by Connant's Quadruple stain schedule. Sections of the stem of normal plants grown from seed in the greenhouse were fixed in FAA and either stained and sectioned as above or sectioned at 20u on a freezing microtome and stained with safranin and fast green.

A cross-section of the normal mature stem of Ipomoea tuba just below the first true leaf shows a phellogen producing a thin-walled phellem several cells in depth on the periphery. Progressing toward the center of the stem from this is located the collenchyma, four or five cells in depth; considerable assimilatory parenchyma containing lactiferous ducts and secretory cells; a uniseriate layer which may be an endodermis, but cannot be strictly identified as such since it lacks Casparian strips and is not a starch sheath; a pericycle; external phloem groups; the cambium, several cells in thickness; a ring of secondary xylem traversed by pith rays; radial rows of primary xylem; and a central pith containing lactiferous ducts and strands of internal phloem (Figure 6). Both internal and external phloem contain well developed sieve tubes and companion cells. The cortex and central parenchyma contain abundant starch and there are many cluster crystals. Bands of fibers occur in the xylem of the older stem. Younger portions of the stem are much the same as to tissues and organization, except that there are no xylem fibers and no phellogen, the stem being covered by a uniseriate epidermis.

A longitudinal section of the normal primordium shows a uniseriate tunica covering the central, homogeneous corpus. Just back of the corpus region is an area of cell elongation and pronounced procambial development. Phloem is difficult to distinguish in the longitudinal section, but xylem elements are easily recognized one to two mm. back of the apex. The origins of lactiferous ducts in both the central and cortical regions may be distinguished at about the same level and many of the parenchyma cells contain large cluster crystals (Figure 7).

The tumors consist largely of parenchymatous tissue with relatively small and, it would seem, inadequate amounts of xylem and phloem. The parenchyma cells are about the same size as those occurring in the central pith of the normal cells. The xylem and phloem cells, on the other hand, are extremely small and there is apparently no cambial activity. The phloem varies from almost completely undifferentiated but elongated cells, which must serve as conducting tissue, to phloem with an apparent organization (in cross section) into sieve tubes and companion cells. However, no sieve plates were observed. The xylem elements are very much shortened with reticulate or spiral thickenings. The conducting tissues are rather regularly arranged in a cylinder around a central pith in each individual swelling of the multiple tumor. An internal phloem differentiates. The available material was not well fixed for cytological purposes, but the nuclei appeared normal. The parenchyma contains cluster crystals especially near the lactiferous ducts and starch grains are particularly abundant in the outermost layers of parenchyma. The lactiferous ducts are not well developed as in the normal stem and there appear to be no functioning secretory cells.

Aside from the proportionately small amount of conductive tissue, the tumors appear histologically surprisingly normal. There are no giant cells, the tissues maintain a regular arrangement, and there is no excessive proliferation of any one tissue. There is simply a general "ground mass" of parenchyma with relatively little xylem and phloem.

The striking histological feature of the tumors is the large number of growing points or primordia most of which fail to continue development. These primordia show a wide range from normality. In normal primordia, apical growth is retarded early and ensuing development and growth is due to intercalary activity in addition to some unlocalized cell division (15). In the tumor primordia, this intercalary growth seems to fail. The procambial strands differentiate into some semblance of a conductive tissue, but no new tissue seems to develop. The meristematic cells enlarge and become parenchymatous (Figures 8_a and 8_b). A phellogen must differentiate in some cases near the surface, but very often the tissue it produces is sloughed off. The surfaces of the primordia often appear to be suberized and some peripheral tissue sloughs off

(Figure 9b).

It would seem that the tumors are formed because the processes of cell elongation and intercalary growth which would normally cause a stem to increase in length are somehow stopped. It is interesting to note in this connection that the growing points in the tumor tissue develop in a phyllotactic sequence (Figure 10).

A recent paper on radiation injury in barley from absorbed P^{32} is of particular interest here (24). It was found that when a meristematic region such as that of the root or stem tip was subjected to a constant, relatively high level of radiation from absorbed P^{32}, cell division ceased and the cells enlarged and took on an abnormally mature appearance.

Smith and Kersten (28) working with seedlings of Vicia faba grown from x-irradiated dry seeds found that there was little elongation in the root and that meristematic tissue such as cambium and pericycle actually degenerated.

In their study of ionizing radiations on the broad bean root, Gray and Scholes (13) found that after high dosages of x-irradiation (three-quarters of a mean lethal dose of x-rays) there was a slowing down of both mitosis and interkinesis in the meristematic region so that the rate of elongation was only about one-fourth normal. However, in the proximal half of the meristematic region cells continued to differentiate at roughly the normal rate but fresh cells were not formed in the distal half to maintain the constant total number of meristematic cells. The effect was "in the main one of mitotic inhibition combined with continued differentiation."

Other workers have observed injury to the meristematic regions in x-irradiated plants. Johnson (18) noted a change in the general aspect of the entire plant as a result of the greater development of lateral branches. This development of the laterals would indicate that the terminal meristem had been injured. In another article (19), she also states that a constant effect of x-rays on seeds and seedlings of Helianthus annuus is the production of fasciation in stems, leaves and flowers.

At Brookhaven National Laboratory it is reported (3) that plants exposed to chronic irradiation in the "gamma field" are often severely stunted or killed. Others show growth abnormalities such as the supernumerary buds in Tradescantia.

While it is well known that plants vary in their response to radiation, we have found no previous record of radiation induced tumors such as the ones described in this paper. However, as has been pointed out, a similar phenomenon has been reported, i.e., a retarding of meristematic growth with continued differentiation; in the present case the phenomenon was carried to such a degree that large tumors resulted. The tumorous plants were limited on the test islands to areas adjacent to the crater site where radioactivity was comparatively high. A careful examination of stands of this species on several islands in each of four atolls revealed no other cases of tumorous Ipomoea plants.

At the time the plants were collected a radiation survey of the collection site was made by Seymour and Kellogg (30). At this time the survey meters recorded 50,000 to 100,000 c/min. at the surface of the soil within the area in question. A conservative estimation of the dosage received by the plants would then be somewhere between 0.1 and 10 rep/week* during August of 1949, seventeen months after the actual bomb tests. Records of earlier levels of radioactivity and of the time when the plants first reestablished themselves are not available.

The tumors themselves were examined both by means of autoradiography and by direct tissue count for radioactivity within them, but nothing more than traces of activity were present in the tissue mass. This is to be expected as the plant is a deep rooted one absorbing very little in the contaminated surface

* Assuming Eav to be 1 mev.

layer. From the work of Jacobsen and Overstreet (17), it is known that fission products are absorbed onto roots but are not translocated in significant quantities to other parts of the plant. Therefore the external radiation which was received was predominatly beta radiation from the contaminated surface layer of the soil.

After careful consideration of all possible causal agents it seems highly probable that radiation is the cause of the tumorous growths on Ipomoea. However, it must be pointed out that we have not attempted to experimentally induce such tumors and that no radiation induced plant tumors have been previously reported in the literature to our knowledge. We feel justified, however, in concluding that the tumorous tissue herein described most nearly resembles radiation damaged tissue.

Acknowledgment. -

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BIBLIOGRAPHY

1. Ann. Rep't. East Malling Res. Sta., 1926 & 1927. "The black currant gall mite on red currants." Vol. 14 & 15: 151-152. 1928.

2. Bennett, C. W., "The relation of viruses to plant tissues." Bot. Rev. 6: 427-473. 1940.

3. Brookhaven National Laboratory, Biology Dep't., Annual Report, July 1, 1951.

4. Braun, A. C., "Recovery of tumor cells from the effects of the tumor-inducing principle in crown gall." Science 113: 651-653. 1951.

5. Brown, N. A. and F. E. Gardner, "Galls produced by plant hormones, including a hormone extracted from Bacterium tumefaciens." Phytopath. 26: 708-713. 1936.

6. Carter, W., "Injuries to plants caused by insect toxins." Bot. Rev. 5: 273-326. 1939.

7. Cook, M. T., "The origin and structure of plant galls." Science 57: 6-14. 1923.

8. Cooper, W. C., "Hormones in relation to root formation on stem cuttings." Plant Physiol. 10: 789-794. 1935.

9. De Ropp, R. S., "The crown gall problem." Bot. Rev. 17: 629-670. 1951.

10. Elliott, C., Manual of Bacterial Plant Pathogens. Williams and Wilkins Company, Baltimore. 1930.

11. Felt, E. P., "The relations of insects and plants in gall production." Ann. Ent. Soc. Amer. 29: 694-700. 1936.

12. Goldberg, E., "Histological responses of cabbage plants grown at different levels of nitrogen to indole (3) acetic acid." Bot. Gaz. 100: 347-369. 1938.

13. Gray, L. H., and M. E. Scholes, "The effect of ionizing radiations on the broad bean root." Brit. Jour. Radiology 24: 1-37. 1951.

14. Greenleaf, W. H., "Induction of polyploidy in Nicotiana." Jour. Heredity 29: 451-464. 1938.

15. Hayward, H. E., The Structure of Economic Plants. The MacMillan Company, N. Y., 1938.

16. Heald, F. D., Manual of Plant Diseases. McGraw-Hill Company, N. Y. 1933.

17. Jacobsen, L., and R. Overstreet, "The uptake by plants of plutonium and some fission products of nuclear fission." Soil Sci. 65: 129-134. 1948.

18. Johnson, E. L., "Effect of x-irradiation upon growth and reproduction of tomato." Plant Physiol. 6: 685-694. 1931.

19. Johnson, E. L., "Effects of x-rays upon growth, development, and oxidizing enzymes of Helianthus annuus." Bot. Gaz. 82: 373-402. 1926.

20. Kelly, S. M., and L. M. Black, "The origin, development and cell structure of a virus tumor of plants." Amer. Jour. Bot. 36: 65-73. 1949.

21. Kraus, E. J., N. A. Brown, and K. C. Hamner, "Histological reactions of bean plants to indoleacetic acid." Bot. Gaz. 98: 370-420. 1936.

22. Link, G. K. K., H. W. Wilcox, and A. D. Link, "Responses of bean and tomato to Phytomonas tumefaciens, P. tumefaciens extracts, b-indoleacetic acid, and wounding. Bot. Gaz. 98: 816-865. 1937.

23. Locke, S. B., A. J. Riker, and B. M. Duggar, "Growth substances and the development of crown gall." Jour. Agr. Res. 57: 21-40. 1938.

24. Mackie, R. W., J. M. Blume, and C. E. Hagen, "Histological changes induced in barley plants by radiation from P^{32}." Amer. Jour. Bot. 39: 229-237. 1952.

25. Painter, R. H., "A study of the cotton flea hopper, Psallus seriatus Reut., with especial reference to its effect on cotton plant tissues." Jour. Agr. Res. 40: 485-516. 1930.

26. Rahn, Otto, "Invisible radiations of organisms." Protoplasma-Monographien 9: 171. 1936.

27. Riker, A. J., E. Spoerl, and A. E. Gutsche, "Some comparisons of bacterial plant galls and of their causal agents." Bot. Rev. 12: 57-82. 1946.

28. Smith, G. F., and H. Kersten, "Root modifications induced in *Vicia faba* by irradiating dry seeds with soft x-rays." Plant Physiol. 16: 159-170. 1941.

29. Struckmeyer, Esther, Plant Growth Substances. Edited by: F. Skoog, Univ. of Wisc. Press. 1951. pp. 167-174.

30. University of Washington Report No. UWFL-23. Sections 4, 5 & 6.

31. Zimmerman, P. W., and A. E. Hitchcock, "Formative effects induced with b-naphthoxyacetic acid." Contr. Boyce Thomp. Inst. 12: 1-14. 1935.

Fig. 1—Ipomoea Tuba, Engebi Island. Area in which abnormal plants were found.

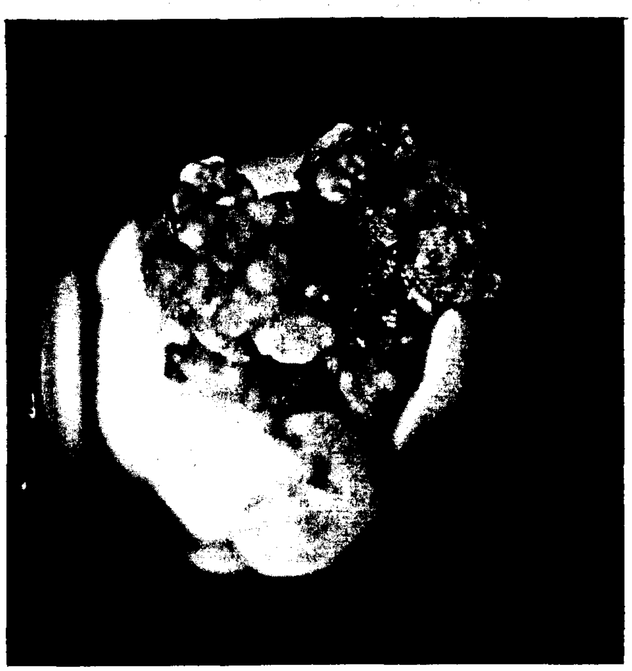
Fig. 2—Normal growth habit of Ipomoea tuba. Engebi Island.

Fig. 3—Tumorous growths at the basal nodes of the stem of Ipomoea tuba. Engebi Island.

Fig. 4—A tumorous Ipomoea plant, Engebi Island. There was very little elongation of the stem, but there is evidence of some recovery in the appearance of regenerated leaves.

Fig. 5a—Tumorous growths; about twice natural size.

Fig. 5b—(1) End of stem showing tumors and regeneration of leaves at the tip. (x 2).



(2) Tumorous mass showing dead primordia in the center and numerous living growing tips on either side.

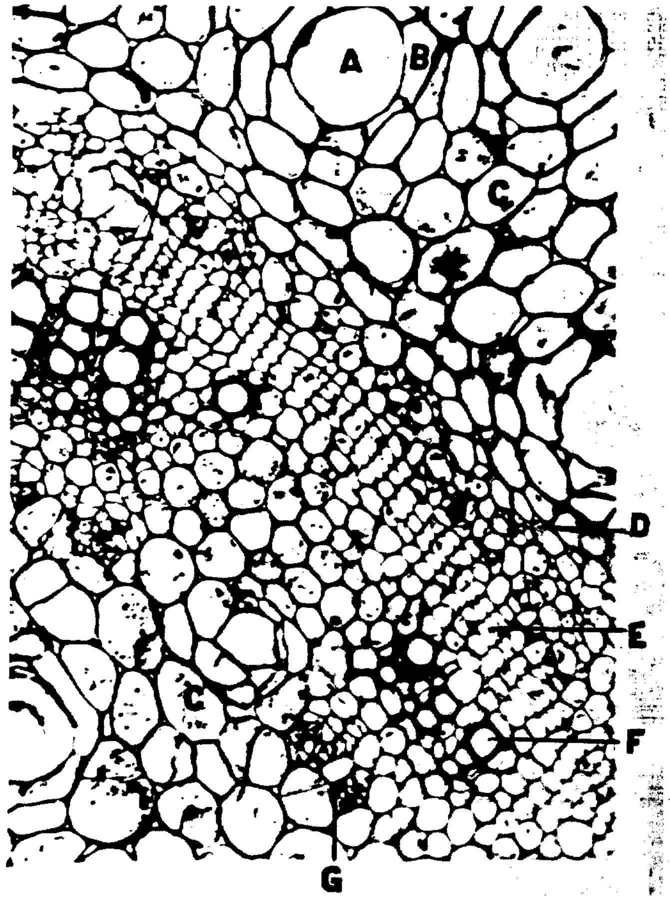


Fig. 6—Photomicrograph of segment of a cross section of the normal stem of Ipomoea tuba. (x 175). A, lacteriferous duct; B, secretory cell; C, parenchyma; D, external phloem; E, cambium; F, xylem; and G, internal phloem.

Fig. 7—Photomicrograph of normal growing tip. (x 145). A, young leaf; B, tunica; and C, corpus.

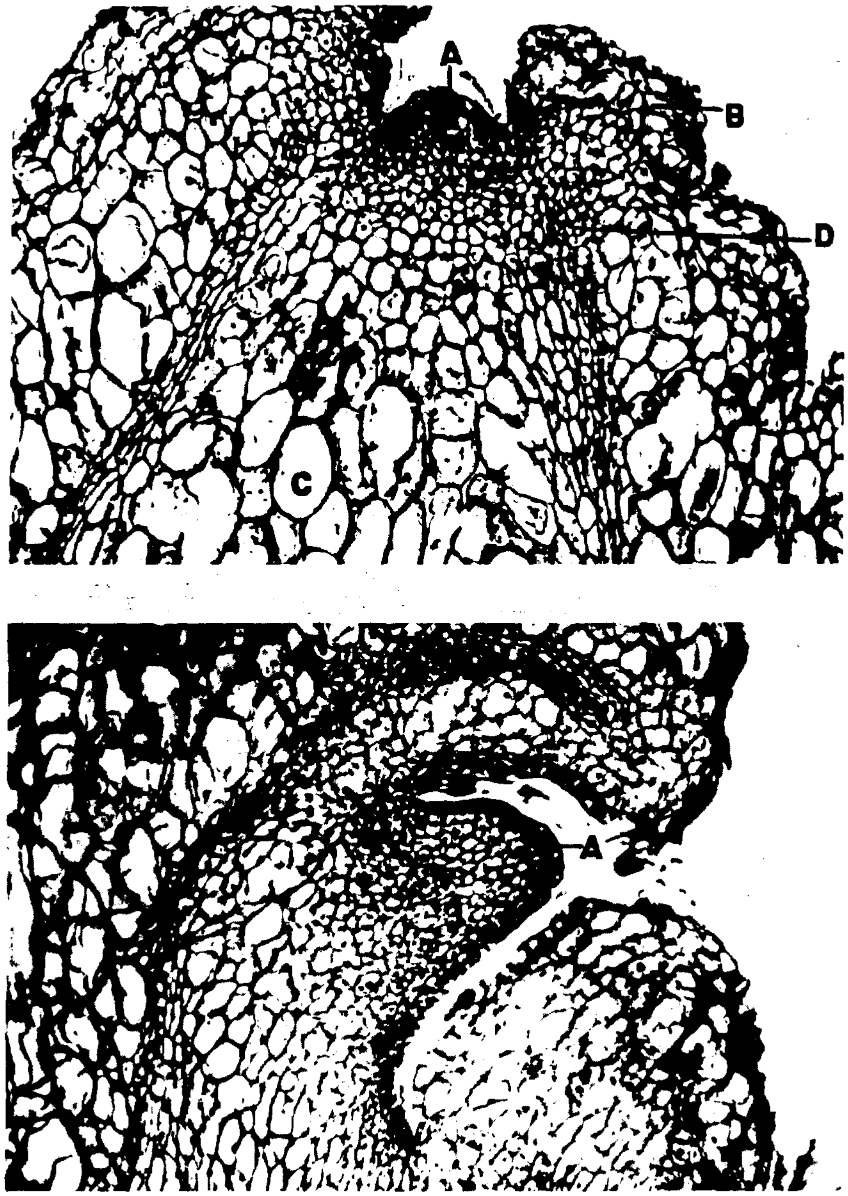


Fig. 8—Photomicrographs of abnormal primordia from tumors. (x 145). Primordia such as the two pictured here were among the more "normal" type found in the tumors. A, suberized surface cells; B, "young leaf"; C, parenchyma; and D, conducting tissue.

Fig. 9a—Photomicrograph of tumor section showing several primordia which had apparently ceased growth.

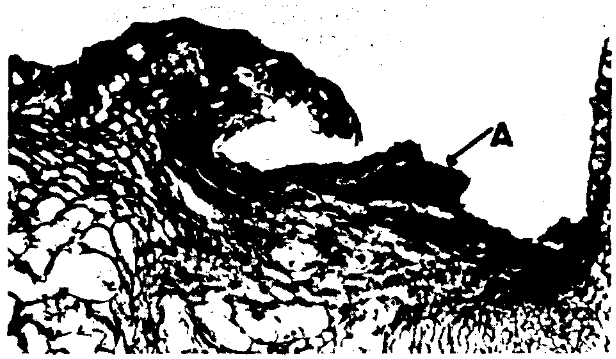


Fig. 9b—Photomicrograph of primordium at "B" above showing A, suberized surface which is being sloughed off. (x 145).

Fig. 10—Surfaces cut from tumors showing phyllotactic sequence of primordia.