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UNITED STATES ATOMIC ENERGY COMMISSION

AECD-3446(App.)

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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April 1953
[TID Issuance Date]

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Work performed under
Contract No. AT(45-1)540.

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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,
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By Susann F. Biddulph and Orlin Biddulph

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

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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

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in "Tissuemat." They were sectioned serially at 15μ 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 20μ on a freezing microtome and stained with safranin and fast green.

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 com-

and ob). 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

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(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

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* 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. -

We wish to acknowledge with gratitude the suggestions made by Dr. E. J. Kraus, Department of Horticulture, Oregon State College, Corvallis, Oregon.

This work has been supported in part by funds contributed by the AEC under subcontract to W-28-094-eng 33, now AT(45-1)540, Applied Fisheries Laboratory.

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

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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.

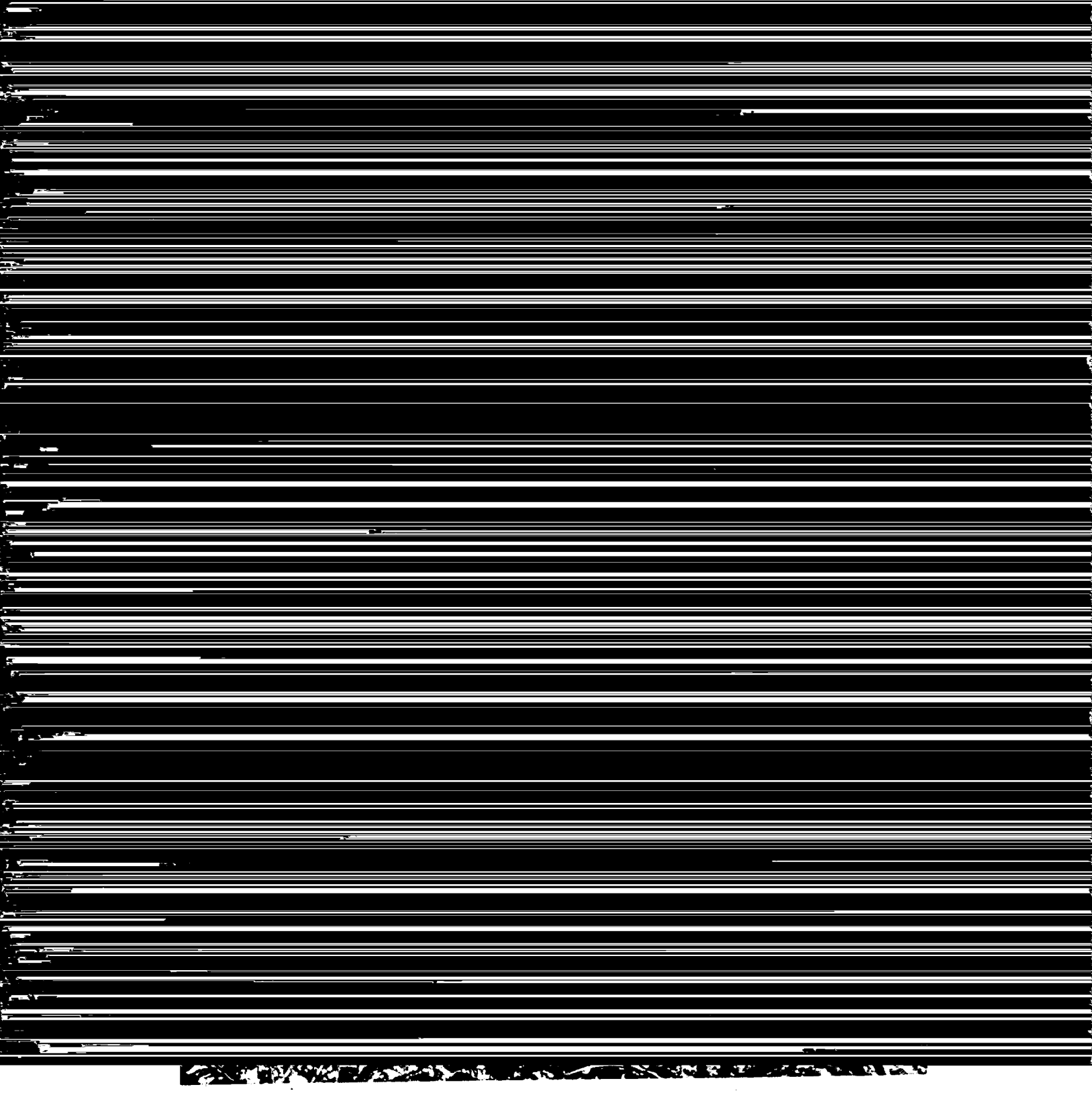

Fig. 4—A tumorous Ipomoea plant, Engebi Island. There was very little elongation of the stem, but there is evidence of some recovery in the appearance of regenerated leaves.

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

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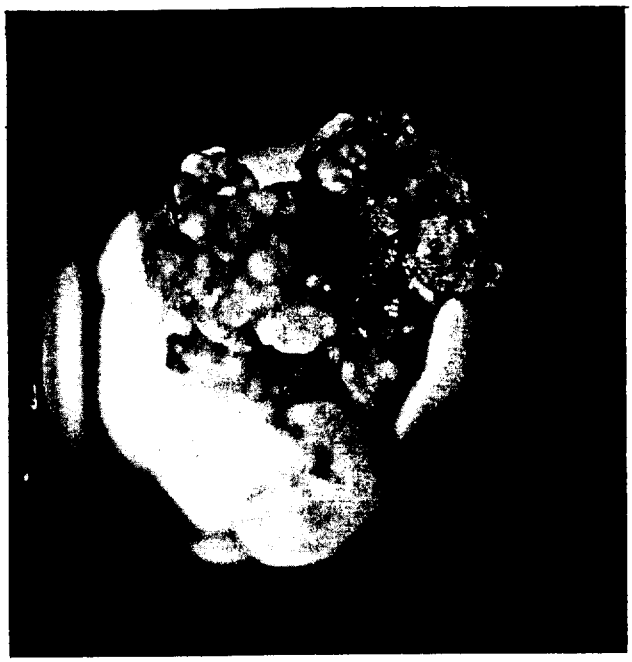
Fig. 5b—(1) End of stem showing tumors and regeneration of leaves at the tip. (x 2).



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

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

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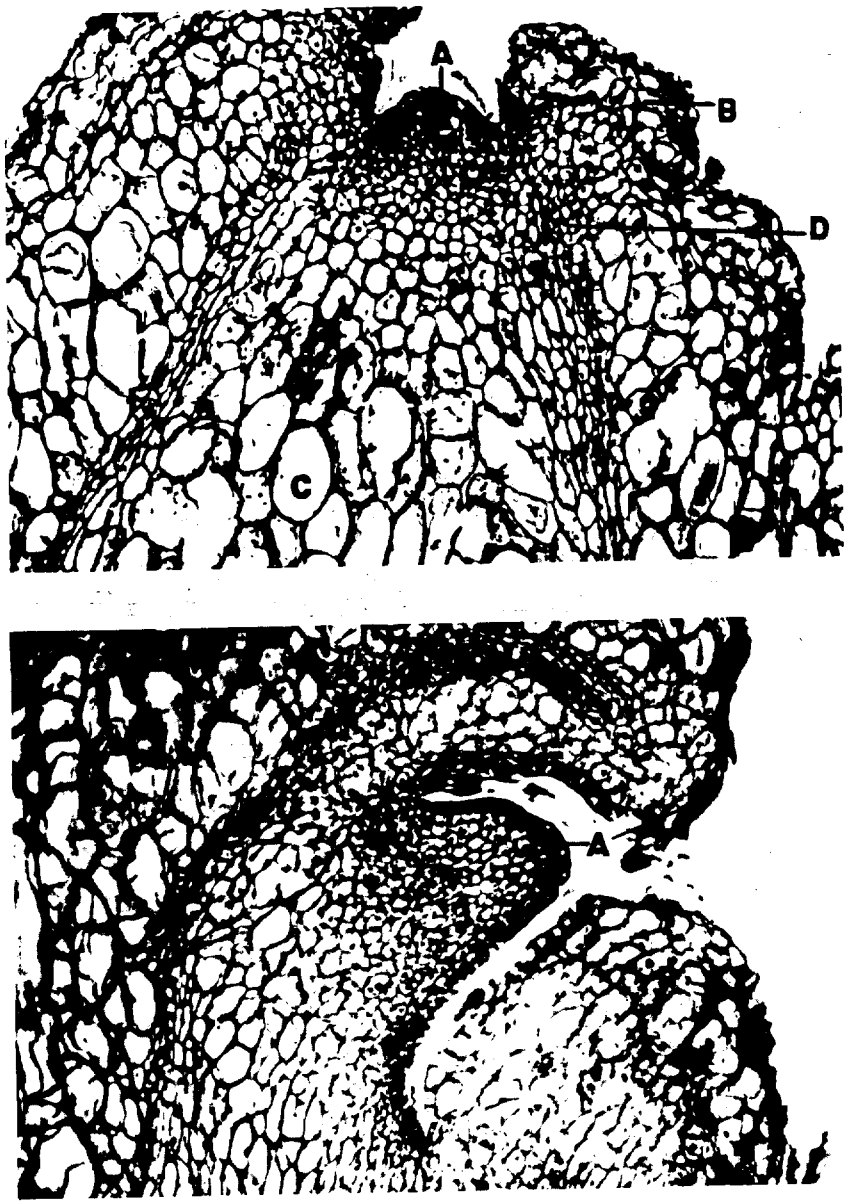

Fig. 8—Photomicrographs of abnormal primordia from tumors. (x 145). Primordia such as the two pictured here were among the more "normal" type found in the tumors. A, suberized surface cells; B, "young leaf"; C, parenchyma; and D, conducting tissue.

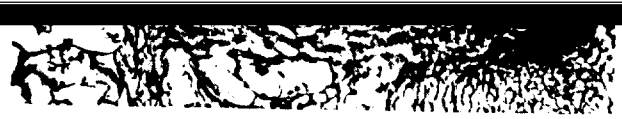

Fig. 9b—Photomicrograph of primordium at "B" above showing A, suberized surface which is being sloughed off. (x 145).

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

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