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A Non-Auxinic Growth-Promoting Factor Present in Crown Gall Tumor Tissue. (21052)

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An abundance of evidence published during the past decade has suggested that crown gall tumor cells proliferate abnormally because they elaborate in greater than regulatory amounts a growth-promoting substance(1-3). Although auxin synthesized by the tumor cell has commonly been regarded as playing an etiological role in abnormal growth of such cells, it is by no means certain that the characteristic behavior of the crown gall tumor cell can be explained solely or even in large part on the basis of high and unregulated auxin levels in those cells. An attempt was therefore made to determine whether, in addition to growth substances of the auxin type, non-auxinic factors capable of stimulating cell proliferation are present in plant tumor tissues.

Methods and materials. In order to investigate this problem it appeared desirable to use as a test object plant cell types that would not proliferate actively in White's culture medium containing auxin. Steward and Caplin(4) have recently reported that mature parenchymatous cells of the potato (*Solanum tuberosum* L.) are not stimulated to active cell division by 2,4-dichlorophenoxyacetic acid. These workers found, however, a synergistic effect to exist between 2,4-D and coconut milk in promoting cell division in the potato parenchyma tissue. Tobacco (*Nicotiana tabacum* L.) pith cells have been reported(5) to enlarge but not to divide under the influence of indole acetic acid. Tobacco pith tissue, which was used in this study, was isolated aseptically from the middle third of greenhouse-grown tobacco plants that were about 3 feet tall. Pith tissue fragments free of internal phloem were cultured either on White's basic medium containing 1% agar(6) or on that medium supplemented with the desired concentration of an auxin and/or tumor extract. The tumor extracts were obtained from sterile crown gall tissue grown in culture. The tumors were harvested during the period of active growth and placed in a Waring Blendor at 4°C. They were thoroughly macerated, pressed through cheesecloth, and centrifuged for 10 minutes at 7,000 r.p.m. The supernatant fluid was incorporated in White's medium in a concentration of 15% by volume in certain experiments described below. The pith tissues were cultured in 50 ml Erlenmeyer flasks containing a total of 14 ml of media. The cultures were maintained at room temperature in diffuse light.

Results. Tobacco pith tissue planted on White's basic medium showed a very limited or, in most instances, no wound healing response even after prolonged incubation, Fig. 1A. In the presence of suitable concentrations of growth substances of the auxin type, however, the volume of the pith tissue more than doubled in a 10-day period, Fig. 1B. The tissue frequently became contorted as if under considerable internal stress. Subsequently, the structure of the tissue itself showed a striking tendency to disintegrate. Enlarged pith cells projected in all directions from what was originally a compact, well organized tissue fragment. In accord with findings reported earlier(5) it was found that proliferation of the pith cells did not occur in the presence of the auxins used. In these experiments naphthalene acetic acid, indole butyric acid, indole acetic acid, and para-

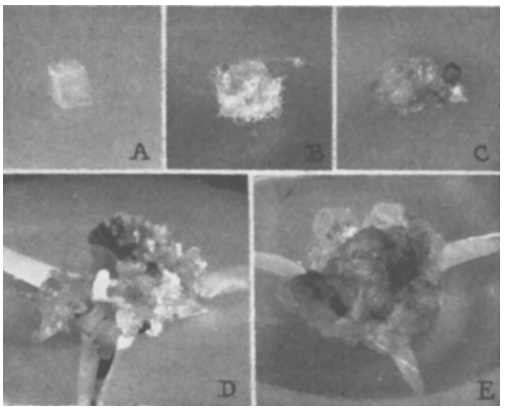

FIG. 1. Tobacco pith tissue planted on: A, White's basic medium; B, White's basic medium containing naphthalene acetic acid in a concentration equal to 1.0 mg/liter; C, White's medium containing 15% *Vinca* tumor tissue extract and naphthalene acetic acid in a concentration of 0.01 mg/liter. The photograph was taken 4 weeks after pith tissue was planted on the medium. D, same tissue shown in C after an additional 4 weeks of growth. Note the organization of leaves of a primitive type arising from the tissue mass. E, White's medium containing 15% *Vinca* tumor extract and naphthalene acetic acid in a concentration of 0.1 mg/liter. The photograph was taken 4 weeks after the pith tissue was planted on the medium. Compare with A and C.

chlorophenoxyacetic acid were tested separately in White's medium in concentrations equal to 100, 10, 1.0, 0.1, 0.01 mg/liter. Naphthalene acetic acid and indole butyric acid were found to be most effective in inducing cell enlargement with resulting contortion of the pith tissue when used in concentrations equal to 10 mg and 1.0 mg/liter. At a concentration equal to 0.1 mg/liter the activity of the 2 growth substances was somewhat less pronounced but still considerable. Indole acetic acid and parachlorophenoxyacetic acid, on the other hand, were found to be far less effective at all concentrations tested.

Since tobacco pith cells did not respond with active cell division to growth substances of the auxin type, an attempt was made to determine whether extracts of crown gall tumor tissue when incorporated in White's basic medium contained a factor or factors that would encourage the active proliferation of tobacco pith cells.

Although in preliminary experiments extracts of crown gall tumors originally isolated from such taxonomically widely separated plant species as *Vinca rosea,* Parthenocissus, and cactus were tested and found to possess varying degrees of biological activity, the studies described below were carried out with the use of extracts obtained from *Vinca rosea* tumors. The *Vinca* tumor extract, which was found to be highly effective, was in all instances incorporated in White's basic medium at a concentration of 15% by volume. Unless otherwise stated the medium was sterilized in an autoclave at 10 lb pressure for 12 minutes. Where used, auxin in the form of naphthalene acetic acid was added aseptically to the medium in the desired concentration following sterilization.

The results of the experiments, which are in part pictured in Fig. 1, demonstrate that within limits a direct correlation exists between the concentration of naphthalene acetic acid in White's culture medium containing 15% tumor extract and the amount of proliferation of the tobacco pith cells. In the presence of a concentration of naphthalene acetic acid equal to 1.0 mg/liter in the tumor-extract-containing culture medium a very active proliferation of the pith cells resulted. At one-tenth this concentration of auxin, proliferation of the pith cells was less pronounced but still considerable (Fig. 1E). It is interesting to note that the growth pattern of the pith tissue in both instances showed, superficially at least, a striking resemblance to tobacco crown gall tumor tissue of the unorganized type grown on White's basic medium. When the concentration of auxin was reduced in the tumor-extract-containing medium to a level equal to 0.01 mg/liter, the tobacco pith tissue grew very slowly. Similar results were obtained in those instances in which auxin was not added to this medium. In further experiments, tumor extract sterilized by filtration with the use of a Selas porcelain filter, 0.03 porosity, and added aseptically to White's basic medium free of auxin failed to encourage proliferation of the tissue. The addition of auxin in a concentration of 0.1 mg or 1 mg/liter to this medium, however, permitted the rapid division of the pith cells. It is likely, therefore, that small amounts of auxin are formed as a result of the sterilization by autoclaving of the tumor extract. This trace

of auxin together with the biologically active factor present in the tumor extract probably accounts for the limited proliferation of pith cells observed in the autoclaved medium to which no external source of auxin was applied.

It thus appears that neither the auxin nor the tumor extract factor can by itself cause normal tobacco pith to proliferate actively. When combined in the proper concentration, however, they are highly effective in accomplishing this end. These experiments have been repeated on 4 different occasions with similar results.

Of interest in these studies was the finding that tobacco pith tissue planted on White's medium containing tumor extract and very low concentrations of auxin grew slowly and in a more or less unorganized manner for a period of about 3-4 weeks, Fig. 1C. Thereafter, numerous small leaf-like structures developed from the tissue mass and in many instances covered the surfaces of the slowly proliferating tissues (Fig. 1D). Such tissues showed a resemblance to tobacco crown gall teratomata. Whether the pith tissue grows in an essentially unorganized manner or as a more or less organized teratoma-like growth appears to depend in large measure on the concentration of auxin present in the culture medium. It has thus been possible to reproduce under controlled conditions growth patterns which resemble superficially at least the 2 morphologically distinct types of crown gall tumors that have recently been described as occurring on tobacco(7). These artificially-stimulated pith tissues are, however, self-limiting and when the externally supplied stimuli are removed their growth commonly ceases entirely or, in some instances, progresses for a time at a very slow rate. Crown gall tumor tissue, on the other hand, is autonomous and is itself capable of synthesizing all of the growth factors necessary for its continued abnormal proliferation.

The biologically active factor found to be present in crown gall tumor tissue extract does not appear to be specifically associated with this plant tumor tissue. Its effect in stimulating the division of tobacco pith cells can be replaced in large part in an auxin-containing culture medium with 15% coconut milk or with an extract of young normal tips of tobacco plants. Since, however, this factor appears to be concerned specifically with cell division, it is likely that it plays a major role in the growth of crown gall tumor cells. Due cognizance should therefore be taken of the tumor extract factor in any consideration of the physiology of development of the crown gall tumor.

Summary. A biologically active factor is present in crown gall tumor tissue extracts which when used in association with an auxin is capable of encouraging the very active proliferation of normal tobacco pith tissue. Neither the factor present in the tumor extract nor the auxin is by itself effective in stimulating the active division of the pith cells.

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