Table 1 compares the quantum yields obtained in this study with those of Weber and Teale as well as with the results of other workers. The values obtained by using Ludox as a standard scatterer show agreement to within 0.02 quantum yield on the average. This corresponds to an average agreement of about 6 percent, the approximate uncertainty associated with a given result.

Generally, glycogen and/or Ludox give quantum yields somewhat higher than those obtained by other workers. The lower values of other workers probably can be traced to one of the following experimental difficulties: improper evaluation of the geometry of the system, materials of questionable purity, or measurements on solutions having too great a concentration (*4*).

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References and Notes

1. G. Weber and F. W. J. Teale, *Trans. Faraday Soc.* **53**, 646 (1957).
2. Ludox is a trade name for an aqueous suspension of silica manufactured by the E. I. duPont de Nemours Company, Wilmington, Del.
3. M. Kasha, *J. Opt. Soc. Am.* **38**, 929 (1948).
4. We are grateful to the National Science Foundation for partial support of this work. We also wish to thank Dr. H. H. Snyder and F. J. Wolter of DuPont for a gift of Ludox.
5. S. B. Sen-Gupta, *J. Indian Chem. Soc.* **15**, 263 (1938).
6. Reported by E. J. Bowen in *Fluorescence of Solutions* (Longmans, Green, New York, 1953), p. 22.
7. S. J. Vavilov, *Z. Physik* **22**, 266 (1924).
8. L. S. Forster and R. Livingston, *J. Chem. Phys.* **20**, 1315 (1952).

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On the Presumed Sterile Induction of Plant Tumors

Abstract. A cooperative reinvestigation of the possibility that filtrates of crown-gall bacterial cultures can induce autonomous tumors in plants has been conducted. The results indicate that the neoplasms formed after treatment do not fulfill the criteria for crown gall since they are not autonomous.

The isolation and characterization of the "tumor-inducing principle" involved in the transformation of normal plant cells into autonomous crown-gall tumor cells (*1*) is important to an understanding of the neoplastic alteration of plants. Klein (*2*) reported that tumor-inducing principle activity for tomato plants was found in filtrates of media containing plant sap in which crown-gall bacteria had grown. This report was confirmed by Manigault *et al.* (*3*) with geranium plants and was extended by Klein and Knupp (*4*) to disks of carrot phloem and by Bender and Brucker (*5*) to tomato, *Datura*, sunflower, and *Kalanchöe* plants.

On methodological and experimental grounds, the report of Klein (*2*) and, on conceptual grounds, the studies of Manigault *et al.* (*3*) were questioned by Braun and Stonier (*6*). Bopp, in a personal communication, reported inconclusive results in attempting to repeat the work of Manigault *et al.*, while Braun and Stonier obtained negative results in three rather large series of experiments. Thomas and Klein (*7*) reported positive results on carrot phloem disks with test solutions produced from precipitated and dialyzed culture media.

In view of the potential importance of the positive reports and the controversy which existed concerning the interpretation of those results, it was felt advisable to join in a cooperative investigation of the problem. This report covers the research conducted by us over a 6-month period, with full access to all available research notes and information.

The positive results on tomato reported by Klein (*2*) were obtained following passage of relatively large volumes of metabolite solutions (about 150 ml) through a single porcelain filter candle having a maximum pore diameter of 1.7 μ. It is clear from results obtained in the cooperative effort as well as from other types of information that this report must be rejected because of bacterial contamination of the test solutions. In this instance the methodological error involved testing too small an aliquot of the total filtrate for sterility. It was found, for example, that even when culture fluids were subjected to centrifugation for 25 minutes prior to filtration through a single candle, the filtrate contained small numbers of bacteria. Of 25 1-ml samples of filtrate tested in one experiment, two samples were contaminated with demonstrably virulent crown-gall bacteria. An equal number of samples of a filtrate obtained by passage through two filter candles of increasing fineness were, on the other hand, uncontaminated. In no instance was it possible to obtain tumors on tomato plants when sterile preparations were applied according to the method described by Klein (*2*).

Kalanchöe, tomato, and *Datura* plants, as well as the phloem tissue of carrot roots, were treated with purified test solutions prepared according to the methods of Thomas and Klein (*7*). There was no response when these preparations were introduced on three successive days into wounded stems of *Kalanchöe* plants. Within 48 to 72 hours after the start of the experiments, localized "swellings" were observed at the points of inoculation in tomato and *Datura*. These did not continue to enlarge beyond a week, a time limit which is characteristic of self-limiting growths resulting from localized areas of irritation.

Similar swellings were observed in tomato but not in *Datura* when wounded controls were treated at the cut stem end above the wound with 1-percent indole-3-butyric acid in lanolin. Wounded control plants showed typical healing responses. Intact plants treated with preparations from media in which avirulent bacteria had grown showed little or no response. On the other hand, tumors initiated by virulent bacteria were still actively growing and had reached massive size after a period of 5 weeks. Disks of carrot phloem tissue treated with these preparations formed small, raised overgrowths which were in no instance of the size or vigor of tumors induced by virulent bacteria. Because of the great variability in the responses of carrot disks treated with test preparations, several different test plants should be examined in such studies.

Since the preparations developed by Thomas and Klein with the use of carrot phloem as a test object did not induce the formation of autonomous neoplasms either on carrot phloem disks or when applied to stems of intact plants of three highly susceptible species, these preparations cannot, on the basis of evidence available, be considered to be the tumor-inducing principle. The concept that sterile induction of crown gall on carrot had been accomplished was derived primarily from etiological considerations in which the timing of the responses, the nature of the pre- and post-treatments, the virulence of the bacteria, and variations in the methods used to obtain active preparations were given paramount consideration. These criteria are not considered adequate for a demonstration of the sterile induction of a crown-gall tumor.

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References and Notes

1. A. C. Braun, *Growth* **11**, 325 (1947).
2. R. M. Klein, *Brookhaven Symposia in Biol.* **6**, 97 (1954).
3. P. Manigault *et al.*, *Ann. inst. Pasteur* **91**, 114 (1956).
4. R. M. Klein and J. L. Knupp, Jr., *Proc. Natl. Acad. Sci. U.S.* **43**, 199 (1957).
5. E. Bender and W. Brucker, *Z. Botan.* **47**, 258 (1959).
6. A. C. Braun and T. Stonier, "Protoplasmatologia," *Handbuch Protoplasmaforsch.* **10**(5a), 1 (1958).
7. A. J. Thomas and R. M. Klein, *Nature* **183**, 113 (1959).
8. This cooperative investigation was conducted at the Rockefeller Institute and was supported in part by grants to one of us (R. M. K.) from the National Institutes of Health (C-4210) and the National Science Foundation (G 8734).

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