Regression of Crown Gall Tumors of Bean Leaves Induced by Glucosamine[1]

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Abstract

Tumors were induced in the primary leaves of beans by inoculation with *Agrobacterium tumefaciens*. Leaves were treated 48 to 72 hours after inoculation by applying 0.1 ml of the test solution over the leaf surfaces. Tumor number, tumor diameter, and leaf size were recorded at the time of treatment and two days later. Glucose and sucrose (0.1 to 0.01 M) stimulated tumor growth. In contrast, tumors treated with glucosamine regressed. Regression was proportional to glucosamine concentration and reached 80 to 90% at 0.1 M. Tumor regression by glucosamine is the first report of successful chemotherapy of a plant tumor.

The regulation of specific enzymes in tumors can be correlated with transformation *per se* as shown by Weber and collaborators in lines of Morris hepatoma (10, 11, 12). Weber has suggested that such multiple effects provide evidence that tumorigenesis is not due to a mutation of a single structural gene but, rather, is a regulatory defect affecting a broad spectrum of biochemical parameters (12). Alternatively, multiple defects could arise from a mutation affecting a single structural gene only if the primary gene product affected was closely coupled to the production of other gene products through normal regulatory mechanisms.

To distinguish among these and other possibilities, experimental approaches would be greatly facilitated by the availability of a system in which tumorigenesis could be reversed. With such a system, both the induction and reversion of the tumorigenic process could be monitored to determine the nature of critical events and the temporal relationships among secondary changes. "Spontaneous" revertants have been reported for several lines of mammalian cells (4, 5, 8). However, the production of these revertants cannot be regulated and they offer no opportunity to monitor the events responsible for reversion.

As one approach to a search for a means to reverse the tumorigenic process, we studied the crown gall tumor of bean induced by the bacterium *Agrobacterium tumefaciens* (7). This system offered advantages of rapid growth, easy accessibility, and the possibility of screening large numbers of antitumor compounds in a short time. By screening several groups of potentially antitumorigenic compounds, one, glucosamine, was found to cause rapid and nearly complete disappearance of already-formed tumors.

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Materials and Methods

Plants of garden bean (*Phaseolus vulgaris* cv. Bountiful) were grown from seed in the greenhouse, 2 plants per 4-inch diam pot, in vermiculite. When the primary leaves entered the phase of rapid expansion (about 7 days after planting), tumors were induced with the bacterium *Agrobacterium tumefaciens* (ATCC Strain 15955) kindly provided by Dr. Ann Matthysse of the Department of Microbiology, Indiana University Medical School, Indianapolis, Indiana. The procedure for inoculation was that of Lippencott and Heberlein (7). Leaves were gently wounded with carborundum and a drop of the bacterial suspension was applied to the surface of the leaf and allowed to dry. Leaves were then rinsed well with deionized water, again allowed to dry and covered with plastic bags. When tumors were 1 mm in diameter (2-3 days after inoculation), 0.1 ml solutions of potential antitumor agents were spread evenly over the leaf surface with a glass rod, and allowed to dry. To determine degree of regression, tumor number and size were monitored before treatment, and 48 hr after treatment, by tracing the outlines on plastic overlays.

Results

Since glucosamine was the first compound found to cause regression of crown gall tumors, attention will be focused on the effects of sugars (Table 1). When tested at concentrations of 50 mM, the following sugars stimulated tumor growth: sucrose, D-glucose, D-fucose, D-fructose, L-arabinose, D-mannose, and L-rhamnose. Lactose and glucose-1-phosphate were without effect. In contrast, marked tumor regression resulted from treatment with D-glucosamine but not from the

TABLE 1. *Effect of sugars, sugar derivatives and related compounds on crown gall tumors induced on bean leaves by Agrobacterium tumefaciens.*

Compound	Tumor Number[1] % of Initial	% of Control
None (Control)	118 ± 17	100
D-Glucosamine	48 ± 6	41
D-Galactosamine	85 ± 4	72
D-Glucose	143 ± 5	121
D-Galactose	125	106
D-Glucose-1-phosphate	127	107
N-Acetylglucosamine	122	103
Lactose	127	107
D-Ribose	115	98
D-Mannitol	108	92
Sucrose	134	113
L-Rhamnose	133 ± 14	113
D-Fructose	170 ± 28	144
L-Arabinose	143	121
D-Mannose	145	123
D-Fucose	153	129
DL-Asparagine	97	82
Ammonium nitrate	109	93

[1] Averages of ten leaves per experiment. Standard deviations are for experiments repeated 3 or more times.

N-acetylated derivative of this sugar. Ammonium nitrate and DL-asparagine were also without effect.

Regression was proportional to glucosamine concentration (Figure 1). Tumoristasis was achieved at 10 mM with nearly complete regression at 120 mM. In separate experiments (data not shown), glucosamine was found to inhibit increase in leaf weight by about 15% at 50 mM but was non-lethal at any of the concentrations tested.

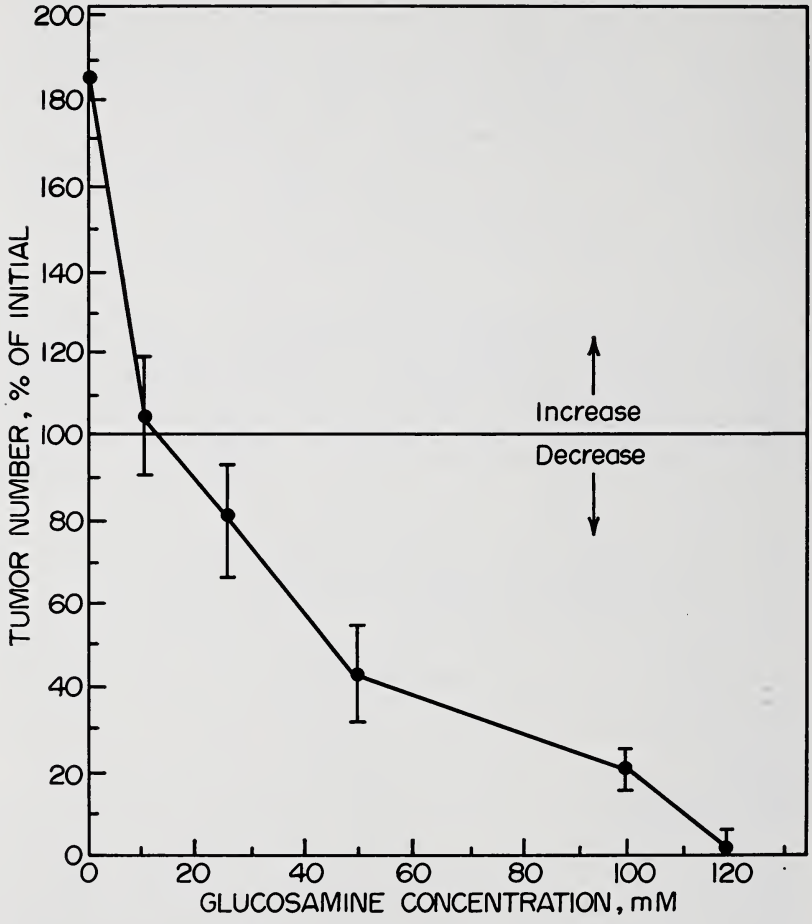

FIGURE 1. *Effect of glucosamine concentation on regression of crown gall tumors induced on bean leaves by* Agrobacterium tumefaciens.

The appearance of tumor bearing leaves before (Fig. 2A) and after (Fig. 2B-D) glucosamine treatment offers striking proof of the effectiveness of the treatment. The only evidence of necrosis after tumor regression is the wound marks of the carborundum inflicted during inoculation (Fig. 2 B-D). Once regressed, the tumors do not reform

and the leaves remain normal in appearance during the life of the plant.

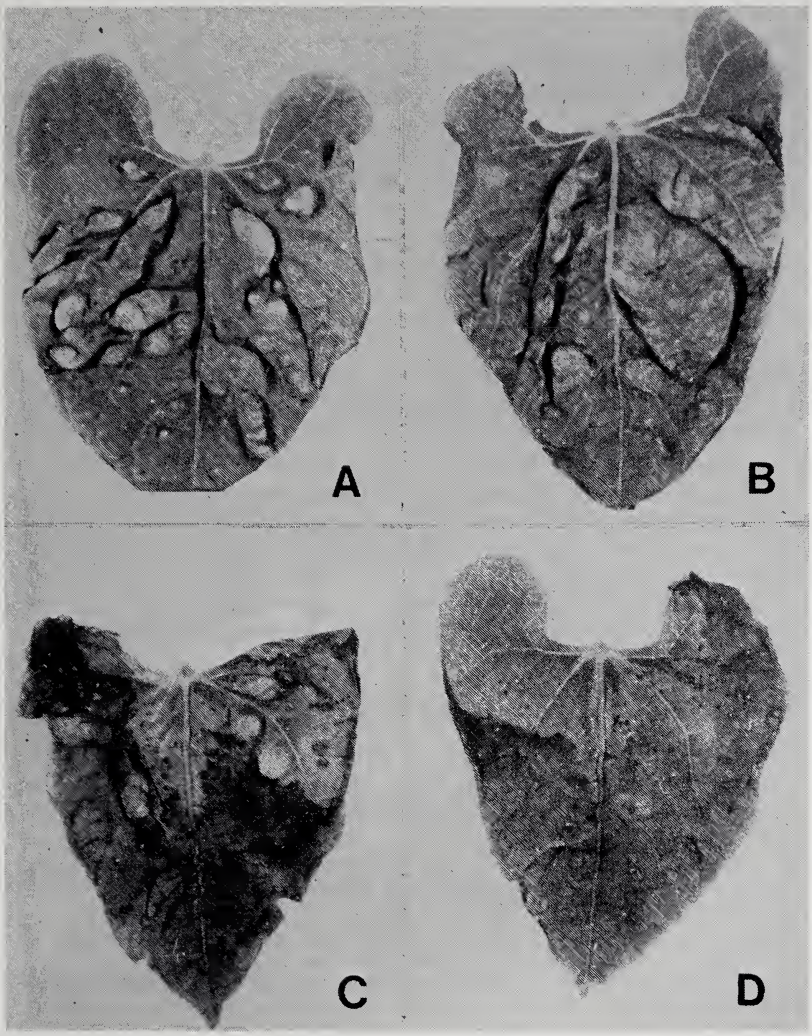

FIGURE 2. *Appearance of bean leaves infected for 72 hours with* Agrobacterium tumefaciens *and subsequently treated for 48 hours with: A. Water. B. 50 mM Glucosamine. C. 100 mM Glucosamine. D. 120 mM Glucosamine.*

Discussion

The regression of bean leaf tumors induced by *Agrobacterium tumefaciens* by the amino sugar, glucosamine, is not elicited by any of the neutral sugars tested including the N-acetyl derivatives of the amino sugar. Inorganic ammonium salts or amino acids did not give the same effect. The effect of the glucosamine in reducing tumor re-

gression is specific and striking. The reversion is virtually complete and the treatment is not cytotoxic at therapeutic doses. In this regard, glucosamine differs from other cancer chemotherapeutic agents which are effective only because they are cytotoxic to the cancer cells. In those forms of the disease where permanent control has been achieved through chemotheraphy, control has resulted from cytotoxicity rather than through a reversal of the tumorigenic process. Thus, glucosamine reversal of crown gall tumors offers a unique opportunity to monitor changes in specific cellular enzymes and constituents of surface membranes during the induction, formation, and reversion stages of solid tumor development.

Various mechanisms have been postulated for the effects of D-glucosamine in animal cell systems (1, 2, 3, 6). However, these do not clearly explain the selective effects of glucosamine on tumor cells. It is possible that this amino sugar is capable of causing repression or induction of specific induced or repressed genetic information, as tyrosine aminotransferase which has been induced by dexamethasone and specifically inhibited by galactosamine in rat liver (9). A similar regulatory repressor role may prove to have great significance in the elucidation of the mechanism of tumorigenesis and its reversal.

The primary significance of the findings rests in the urgent need for a mechanistic framework within which the biochemical basis of tumorigensis and/or malignant transformation can be understood and explained. By "curing" a plant tumor and understanding how this cure has been achieved, it may be possible to design similar cures for solid mammalian tumors based on common underlying principles.

Literature Cited

1. BEKESI, J. G., and R. J. WINZLER. 1969. The effect of D-glucosamine on the adenine and uridine nucleotides of sarcoma 180 ascites tumor cells. J. Biol. Chem. 244:5663-5668.

2. BEKESI, J. G., Z. MOLNAR, and R. J. WINZLER. 1969. Inhibitory effect of D-glucosamine ond other sugar analogs on the viability and transplantability of ascites tumor cells. Cancer Res. 29:353-359.

3. BOSMANN, H. B. 1971. Inhibition of protein, glycoprotein, RNA and DNA synthesis by D-glucosamine and other sugars in mouse leukemic cells L-517BY and selective inhibition in SV 3T3 compared with 3T3 cells. Biochim. Biophys. Acta 240:74-93.

4. BRADY, R. O., and P. T. MORA. 1970. Alterations in ganglioside pattern and synthesis in SV_{40}-and polyoma virus-transformed mouse cells. Proc. Nat. Acad. Sci. U.S.A. 218:308-319.

5. HAKOMORI, S. and W. T. MURIKAMI. 1968. Glycolipids of hamster fibroblasts and derived malignant transformed cells. Proc. Nat. Acad. Sci. U.S.A. 59:254-261.

6. HATANKA, M. 1974. Transport of sugars in tumor cell membranes. Biochim. Biophys. Acta. 355:74-104.

7. LIPPENCOTT, J. A. and G. T. HEBERLEIN. 1965. The induction of leaf tumors by *Agrobacterium tumefaciens*. Amer. J. Bot. 52:396-403.

8. MORA, P. T., R. O. BRADY, R. M. BRADLEY and V. W. MCFARLAND. 1969. Gangliosides in DNA virus-transformed and spontaneously transformed malignant mouse cell lines. Proc. Nat. Acad. Sci. U.S.A. 63:1290-1296.

9. REYNOLDS, R. D., and W. REUTTER. 1974. Inhibition of induction of rat liver tyrpsine aminotransferase by D-galactosamine. Role of uridine triphosphate. J. Biol. Chem. 248:1562-1567.

10. WEBER, G. 1974. The Biochemical Strategy of the Cancer Cell. Academic Press, New York.

11. WEBER, G., and M. A. LEA. 1967. The molecular correlation concept: An experimental and conceptual method in cancer research, p. 523-578. *In* H. BUSCH (ed.). Methods in Cancer Research. Vol. 2. Academic Press, New York.

12. WEBER, G., A. TREVISANI, and P. C. HEINRICH. 1974. Operation of pleotropic control in hormonal regulation and in neoplasia, p. 11-41. *In* G. WEBER (ed.). Advances in Enzyme Regulation. Vol. 12. Pergamon Press, Oxford.