## Tumour Induction by Bacteria-Free Crown-Gall Tissue

Doris M. McEwen
University of Western Ontario

The cancerous response of bacteria-free crown-gall tissue has been established by continued proliferation free from the causal organism or external source of auxin, and by experimental implanting. However, the action of an implant on a healthy host has remained in question, since the implant might effect a cancerous response (de Ropp[1-5]), no response (White and Braun[2]), or a temporary stimulatory response (de Ropp[1-5]).

In order that the response of the implant and host tissues could be observed more clearly, bacteria-free crown-gall tissue was isolated from primary galls of red and white varieties of **Beta vulgaris** and cultured on White's media[4]. To aid in obtaining tumour cultures free from **Agrobacterium tumefaciens,** penicillin and streptomycin were used but not found effective. The tumour tissue failed to proliferate in the rapid manner of autonomous sunflower tumour tissue, so that only the latter was used for implanting.

This sunflower tumour tissue was grafted in vitro to healthy stem segments isolated from sunflower seedlings. From two to seven weeks later, the growing tumour implant was removed easily and the host tissue retained for subsequent growth. In all cases where the implant had died, the hosts were likewise retained. Control stem segments were left free of tumour tissue or else grafted with healthy stem segments.

The tumour implant, which continued to grow in the same manner as it had on nutrient agar, could be distinguished from the host by its characteristic nodular texture and fawn colour. It was removed easily from the host callus because of sparse areas of weak fusion. The host stocks which supported such implants exhibited varying degrees of a stimulatory response beyond that of control stems: the production of abnormally swollen roots, of abnormal basal callus and apical callus, and of overall cortical proliferation. This response was similar to that of sunflower tissue exposed to high concentrations of an auxin (de Ropp[6]). However, on continued culture after removal of the implant the host stocks showed an earlier necrosis than controls, and no further proliferation.

In cases where the implant died after about two days, the host exhibited a limited stimulatory response and a delayed necrosis comparable with that of control stems and stems treated with low concentrations of growth hormones (de

Ropp[7]). However, five percent of the stems bearing dead implants produced a tumorous protuberance which arose from the apical callus of the host two weeks after implanting. At first, these protuberances were greenish-white, smooth- surfaced spheres resembling the host callus, but they rapidly grew into large tumors which showed the cream colour and nodular growth habit of bacteria-free crown-gall tumour tissue. Further growth-behavior of this new tumor tissue was identical with that of autonomous crown-gall tumour tissue.

As evidenced by the above, the action of a growing tumour implant on a healthy host effects a temporary stimulatory response which does not continue in the absence of the implant and which hastens necrosis. In view of the parallel stimulatory response of healthy tissue to high concentrations of auxin, as well as direct (Kulescha and Gautheret[8]) and indirect evidence (Hildebrandt and Riker[9]; de Ropp[7]; Gautheret[10]; Struckmeyer, Hildebrandt, and Riker[11]) of the production of auxin by bacteria-free tumour tissue, this response could be explained on the basis of the auxin supplied by the tumour implant. However, in certain cases where the implant was short-lived the host subsequently produced a tumour which exhibited all the characteristics of autonomous crown-gall tumour tissue. The experimental tumourization of healthy tissue by supply of auxin (Gautheret[10]) has only brought about a response intermediate between that of normal and crown-gall tumour tissue. However, the tumourization of healthy tissue observed here, through response to a short-lived implant, has been positive in nature. Various substances, including auxin, diffusing from the implant during its survival may bring about the production of such new tumour tissue.

**References**

1. de Ropp, R. S., Amer. J. Bot., **34**: 248, 1947.
2. White, P. R., and Braun, A. C., Cancer Res., **2**: 597, 1942.
3. de Ropp, R. S., Bull. Torrey Bot. Club, **75**: 45, 1948.
4. White P. R., "A Handbook of Plant Tissue Culture", J. Cattell Press, Lancaster, Penna., 1943.
5. de Ropp, R. S., Bot. Rev., **17**: 629, 1951.
6. de Ropp, R. S., Amer. J. Bot., **35**: 372, 1948.
7. de Ropp, R. S., Amer. J. Bot., **34**: 53, 1947.
8. Kulescha, Z., and Gautheret, R. J., Comp. Rend. Acad. Sci., **227**: 292, 1948.
9. Hildebrandt, A. C., and Riker, A. J., Amer. J. Bot., **34**: 421, 1947.
10. Gautheret, R. J., Compt. Rend. Soc. Biol., **142**: 774, 1948.
11. Struckmeyer, B. E., Hildebrandt, A. C., and Riker, A. J., Amer. J. Bot., **36**: 491, 1947.