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AGONIST AND/OR ANTAGONIST EFFECTS OF PLANT HORMONES AND AN ANTICANCER ALKALOID ON PLANT DNA STRUCTURE AND ACTIVITY

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Using a biochemical assay system (Oncotest) (1) we have shown that DNAs from cancerous mammalian (2) and plant cells (3) are destabilized compared to DNA from healthy cells. Cancer DNAs susceptible to the action of carcinogens or other different compounds exhibit *in vitro* and *in vivo* a high template activity in comparison to DNAs from healthy cells (2, 3). We have also shown that alstonine, a plant alkaloid prepared in our laboratory (4), which selectively binds to DNA from cancer cells prevents DNA *in vitro* synthesis as well as cancer cells *in vivo* multiplication in animals (2) and plants (5). It has a slight effect on DNA from healthy cells. We describe here the effects of auxin (IAA) and kinetin (K) (two cell growth and division hormones in higher plant species) and that of alstonine (BG-8) on *in vitro* synthesis and strand separation of DNAs isolated from cancerous, habituated and healthy plant cells cultured *in vitro*.

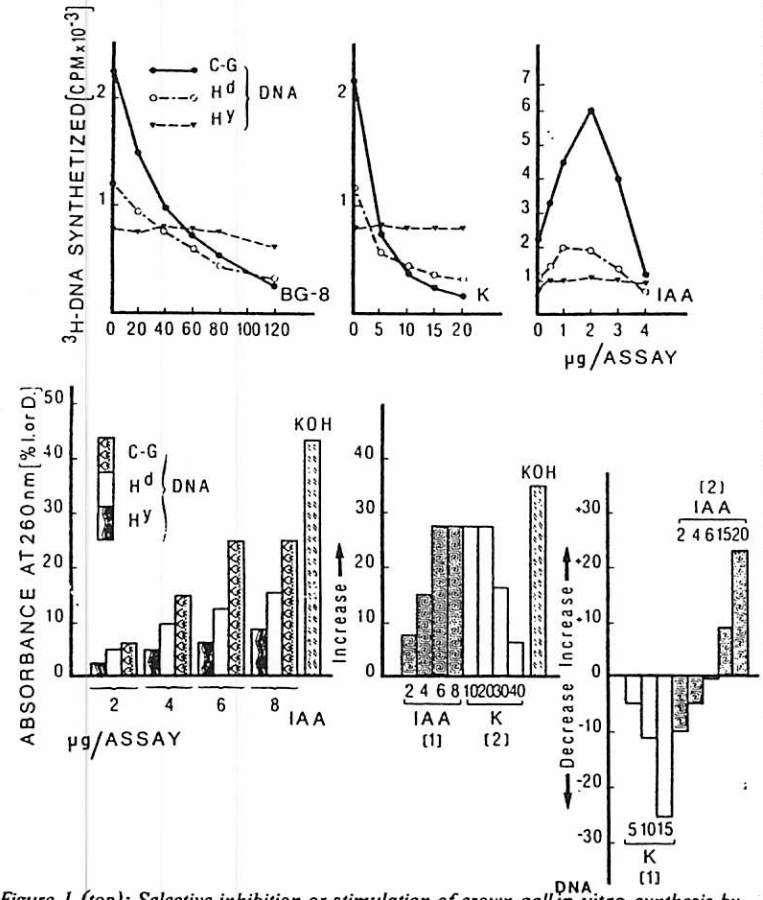

Figure 1 (top): Selective inhibition or stimulation of crown-gall in vitro synthesis by BG-8 and K or IAA respectively. Crown-gall (C-G), habituated (H^d) and healthy (HY) DNAs were used as templates in the presence of these three compounds. Figure 2 (bottom): Crown-gall (C-G), habituated (H^d) and healthy (HY) DNAs strand separation in the presence of K and IAA. UV absorbance (expressed in % increase or decrease) of DNAs was measured in the absence or presence of different concentrations of one (IAA) or two compounds (successively IAA and K or K and IAA) added to the blank (buffer solution, see 1) and the DNA solution.

Methods: Hormone-autotrophic crown-gall tumors (6) and primary normal hormone-dependent callus were derived from *Nicotiana tabacum* and *Ampelopsis hederacea*. The habituated cell line was spontaneously derived from the normal hormone-requiring callus of *A. hederacea* and no longer required the presence of hormones for growth *in vitro*, behaving as do real plant tumor cells. The tissues were kindly supplied by Dr. Aaron and aseptically cultured (6). DNAs from healthy, cancerous and habituated cells were isolated and purified by phenol and chloroform (3). The amount of DNA and the hyperchromic effect (compared with that obtained with KOH) were determined by UV absorbance at 260 nm (1, 2). Conditions for the *in vitro* template DNA assay system have been described (1). The amount of acid-precipitable ^{3}H-DNA synthesized was measured in the absence or presence of the compounds used.

Results: As shown in figure 1, the amount of DNA synthesized *in vitro* on DNA template isolated from crown-gall tissues was always higher than that synthesized in the presence of healthy tissues DNA. Template activity of habituated tissues DNA lay in between both. BG-8 differentially inhibited both the *in vitro* synthesis of crown-gall and habituated tissue DNA, healthy DNA being practically insensitive. Similar results were obtained with K. In contrast, IAA selectively stimulated crown-gall DNA synthesis when used at low doses while high doses were inhibitory. This corroborates and explains the known effect of IAA observed *in vivo*. The selective binding of BG-8 to initiation sites of destabilized DNA only from plant (5), as well as mammalian cancer DNA (4), explains its inhibiting effects on *in vitro* cancer DNA synthesis thence on *in vivo* cancer cell multiplication (in preparation). It is important to note the antagonist effects of IAA and K which in the Oncotest act in opposite ways: IAA behaves as a carcinogen and stimulates *in vitro* crown gall DNA synthesis (3) and *in vivo* crown gall cells multiplication (3, 7) while K does not. As shown in figure 2, there is *in vitro* a progressive increase in UV absorbance for crown-gall DNA but not for healthy DNA in the presence of increasing concentrations of IAA while habituated cells DNA presents intermediate values, facts closely related to the extent of DNA destabilization and consequently DNA replication. Crown-gall DNA, the most destabilized one, the most susceptible to a further destabilization and the best template for DNA replication was used to show the antagonism between IAA and K. In the presence of IAA, crown-gall DNA undergoes a very strong DNA strand separation which may be antagonized by the addition of K. Conversely, there is a decrease in UV absorbance when crown-gall DNA solution contains K or BG-8; an effect sup-

pressed by IAA. This correlates with DNA *in vitro* synthesis. According to the order of addition of these two hormones, we observed a hypo- or hyperchromic effect. The reversible competition between IAA and K on crown-gall DNA hyperchromicity is not observed when healthy DNA samples are used. These results may be compared with data (8) which indicate that the sequence of hormone applications determines the fate of plant cultured cells. Results concerning the effects of BG-8 alone and together with growth-regulating substances IAA and K on crown-gall cells cultured *in vitro* will be described elsewhere.

Conclusion: The results presented show that there is a correlation between *in vitro* DNA strand local separation, DNA synthesis and multiplication rate of crown-gall and healthy plant cells cultured *in vitro*. These observations support the concept that habituation as well as the neoplastic state of cells may be a reversible process. Interaction between IAA and K in association with some molecules such as BG-8 may be involved in the regulation of DNA chain opening or closing thus contributing to the transformation process of a normal cell to cancerous one or of a cancerous cell to a normal one.

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