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ONCOTEST: A DNA ASSAY SYSTEM FOR THE SCREENING OF CARCINOGENIC SUBSTANCES

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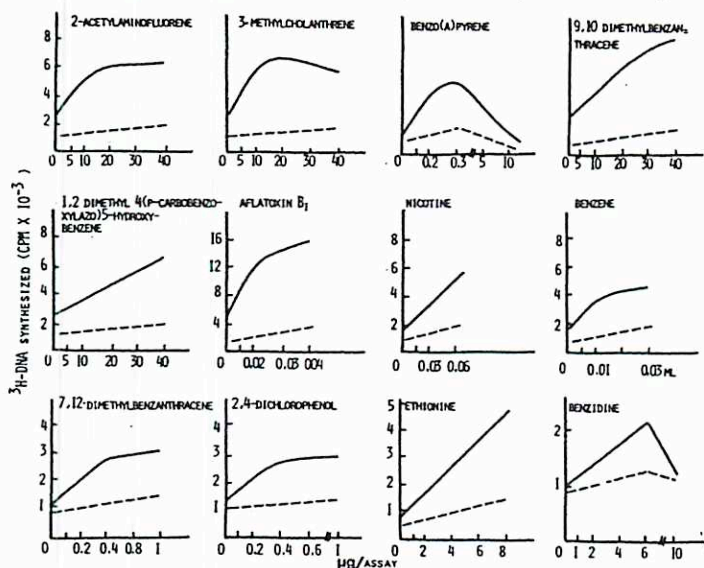
We describe a very rapid, sensitive and economic assay system (Oncotest) using purified DNA(s) from both cancerous and healthy human tissues (lung, breast, ovary and brain) and DNA dependent-DNA polymerase I, partly purified from *Escherichia coli* for detecting potential carcinogens. The amount of *in vitro* synthesized radioactive DNA as acid precipitable product is measured under strictly identical experimental conditions in the absence or presence of various concentrations of compounds to be screened. All known carcinogens and several drugs we tested strongly stimulate the synthesis of cancer DNA and only slightly that of DNA from healthy tissues. The results can be obtained within 1 to 4 h.



Dose-response curves of known carcinogens. Ordinate (^{3}H-DNA in vitro *synthesis). —— cancer DNA; - - - - - DNA from healthy tissues. Short vertical marks along the abscissae indicate concentrations used.*

Methods: DNA(s) from cancerous and healthy human tissues were isolated and purified by phenol and chloroform methods as previously described (1). DNA dependent-DNA polymerase I from *E. coli* was partly purified as described in *Biochemical Genetics* (2). Incubation mixture contains per 0.15 ml: tris-HCl buffer (pH 7.65): 25 µmoles; $MgCl_2$ 2 µmoles; four deoxyribonucleoside-5'-triphosphates, each 5 nanomoles (+ labelled TTP, 50 000 cpm); 0.5 to 1 µg of purified cancer or normal DNA; 0 to 60 µg of compounds to be tested; 40–80 µg of DNA dependent-DNA polymerase I added as the last constituent of the mixture. Incubation for 10 min at 36°C. Trichloroacetic acid (5% final concentration) is added to the incubation mixture and the acid precipitable product is filtered on a glass GF/C millipore filter, washed and dried. The radioactivity is measured with a Packard liquid spectrometer (Prias). In each assay, DNA(s) from cancerous and healthy tissues must be used.

Results and discussion: Incubated with the necessary components and DNA template from cancerous tissues for *in vitro* DNA synthesis, well known carcinogens strongly stimulate DNA synthesis as acid-precipitable radioactive product. When incubated under identical conditions with DNA from the corresponding healthy tissues, these same carcinogens only weakly stimulated DNA synthesis. Dose-response curves for known carcinogens are given in the figure. Their stimulating action is always several times higher on the four types of cancer DNA(s) than on the corresponding normal DNA(s). Among carcinogens tested some can be detected with 0.01 µg per assay (aflatoxin B_1), others act as carcinogens at low concentrations while at higher they are toxic and inhibit DNA dependent DNA polymerase. To confirm that carcinogens distinguish cancer DNA(s) from normal DNA(s) we showed that ethionine, a potent carcinogen increases to 10–15% the U.V. absorbance at 260 nm of all cancer DNA(s) tested and only 3% that of normal DNA(s) (complete results not presented here). We emphasize that in the presence of alkali both cancer and normal DNA(s) show U.V. absorbance increased to 25–30%. These data suggest that carcinogens bring about separation of double stranded regions of cancer DNA thus offering more template to DNA dependent DNA polymerase I. Several chemotherapeutic drugs submitted to our biochemical test behave like authentic carcinogens: adriamycin, daunorubicin, thio-Tepa, endoxan (cyclophosphamide), 5 Fu, rifampicin, vinblastine, vincristine, bleomycin etc. Also steroids, used at non-physiological concentrations, act as potential carcinogens. Actinomycin D and bleomycin, which are inactive as mutagens in the *Salmonella* assay systems (3), behave in the Oncotest as carcinogens, which is comparable to the carcinogenic effect of these drugs in animals (4). Among the 200 compounds submitted to the Oncotest, 35% were found to act as well known carcinogens. Substances like pure saccharin, cholesterol . . . are indifferent during DNA synthesis while some (paraquat, diquat . . .) are toxic for DNA dependent DNA polymerase. Substances which specifically inhibit cancer DNA replication with practically no effect on healthy DNA synthesis should be considered as specifically anti-cancer drugs.

Discussion: Results obtained by the Oncotest suggest that there must exist some molecular mechanism by which carcinogens induce modifications in cancerous DNA which is particularly susceptible to these agents. These observations imply that carcinogens may further act on DNA in cancerous cells thus accelerating their multiplication as they do in plant tumorous cells (5). In these studies Oncotest may be an useful assay for detection of carcinogens and compounds able of distinguishing and selectively killing cancerous cells.

1. Beljanski, M., *et al.*, (1974) *Proc. Nat. Acad. Sci. USA*, 71, 1585-1589
2. Beljanski, M. and Beljanski, M.S. (1974) *Biochem. Genet.*, 12, 163-179
3. Benedict, W.F., *et al.*, (1977) *Cancer Res.*, 37, 2209-2213
4. Sieber, S.M. (1975) *Cancer Chemother. Rep.*, 39, 915-918
5. Le Goff, L. and Beljanski, M. (1979) *IRCS Med. Sci.*, 7, 475