THE CARCINOGEN N-ACETOXY-ACETYLAMINOFLUORENE REACTS PREFERENTIALLY WITH A CONTROL REGION OF THE INTRACELLULAR SIMIAN VIRUS 40 CHROMOSOME

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The chromosome of Simian Virus 40 (SV40) is well suited for studies of the interaction of chemical carcinogens with chromatin. Like chromatin of eukaryotic cells, it is organised into nucleosomes. We have investigated the binding of the ultimate carcinogen N-acetoxy-acetylaminofluorene (AAAF) to specific regions of the SV40 chromosome in situ in the intact infected cell. SV40-infected cells late in the lytic cycle were incubated with ^{3}H-AAAF. SV40 DNA was extracted, digested with restriction enzymes HaeIII and KpnI, and radioactivity in each DNA fragment determined. The results indicated that a stretch of DNA near the origin of replication of the intracellular SV40 chromosome was more susceptible to attack by AAAF than the rest of the SV40 genome. When naked DNA was labeled with ^{3}H-AAAF in vitro no hyperreactive region was seen. The hyperreactive region may represent a stretch of DNA which is nucleosome-free or has another unusual chromosomal structure.
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HYPERTROPHY AND HYPERPLASIA OF MOUSE EPIDERMIS INDUCED BY 3-METHYLCHOLANTHRENE AND PHORBOL ESTER ARE STRAIN DEPENDENT AND MAY CORRELATE WITH SUSCEPTIBILITY TO TUMOR INDUCTION

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In two-stage carcinogenesis models (tumor-"initiation" and -"promotion") of mouse skin cell proliferation and subsequent epidermal hyperplasia (HP) are implicated in the concept of tumor promotion. The possible importance of cell hypertrophy (HT), however, is unclear. Epidermal lesions induced by 3-methylcholanthrene (MCA) and by 12-0-tetradecanoylphorbol-13-acetate (TPA) were analyzed by means of morphometry in mouse strains differing in susceptibility to tumor induction. In all strains MCA, a complete carcinogen (i.e. which in addition to a tumor initiating-property also possesses promotorlike activity) caused epidermal thickening peaking between the 2nd and 5th day after a single treatment. This thickening was due to both increase in average cell size (HT) and an increase in the number of cells (HP) per unit of length of interfollicular epidermis. After MCA HP as well as HT were significantly greater in carcinogen susceptible BALB/c (HP 135 %, HT 300 %) and C57BL (HP 140 %, HT 330 %) than in more resistant DBA (HP 120 %, HT 150 %) and AKR (HP 120 &, HT 175%) mice. From these values it appears that HT was more marked than HP and that the difference in the hypertrophiogenic effect of MCA between high and low responders was more obvious than the difference in the hyperplasiogenic effect of the carcinogen. Similar strain-specific differences in HP as well as HT between high responders and low responders of tumorigenesis were noted using the tumor promoting agent TPA which needs no metabolic conversion for its action. The present studies rise the possibility that - in addition to possible differences in the initiation phase and/or DNA repair processes - strain-specific differences of epidermal reactions in the promoting phase might be important in susceptibility to tumor induction.

HEAVY WATER : EFFECTS ON PROLIFERATION OF NORMAL AND NEOPLASTIC CELLS IN MICE

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In 1938, Barbour and Allen observed reduced growth rates of transplantable carcinoma or lymphosarcoma in nine deuterated mice. In the fifties and sixties, it appeared from the results of several similar experiments that moderate body deuteration of mice and rats may slow down tumor growth and sometimes prolong survival. We found only one study focussing on the combined effects of heavy water and cytostatic drugs on a small number of mice inoculated with Krebs-2 ascites tumor; in spite of reduced tumor growth rates, these animals died earlier than the controls.
In the present study, young adult DBA/2 mice bearing transplantable tumors were treated with different concentrations of heavy water and with repeated injections of methotrexate (MTX). We used several lymphoid tumors, and the mastocytoma P 815.

Generally, there was a significant and reproducible increase in survival time of deuterated, tumor-bearing mice treated with MTX. Further, it appeared that deuteration at an effective antineoplastic level does not markedly affect proliferation of normal cell renewal systems, as examined by the incorporation of tritiated thymidine into the DNA of bone marrow and small intestine following prolonged exposure of normal mice to moderately deuterated drinking water.
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HYDANTOIN INDUCED LYMPHOMAS : BENIGN OR MALIGNANT ?

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Hydantoin derivatives are the most commonly used antiepileptic drugs with well-known, mostly harmless side-effects. One of the most controversial complications of long-term hydantoin therapy is the appearance of lymphomas. They are mostly multiple, self-limiting with signs of an allergic reaction and complete remission after withdrawal of the drug. However there is an increasing number of observations of clear-cut malignant lymphomas after long-term hydantoin therapy, progressing after discontinuation of the drug. We have seen 8 cases of malignant lymphomas after antiepileptic treatment with predominantly hydantoin derivatives of 7-23 years duration. 2 are Hodgkin's, 6 Non-Hodgkin's lymphomas of various histological types. We have added 4 new cases to those already presented (Schweiz. med. Wschr. 105, 1735, 1975). The only common characteristic of these 8 cases - as in most others reported - is the unalterable fatal outcome of the disease despite all therapeutic measures. There is no conformity in type or course of the epilepsy nor in the clinical or histological presentation of the ensuing lymphomas. Hydantoin derivatives have a well documented carcinogenicity. Long-term hydantoin treatment may occasionally lead to the development of malignant lymphomas, which is only seldom preceded by a typical hydantoin lymphadenopathy (documented in one of our cases). There is no way to identify potential candidates for this detrimental, irreversible complication of an otherwise well tolerated routine drug therapy. Close observation of hydantoin-treated epileptics and central registration of all cases of hydantoin-induced lymphomas are warranted.

TUMOR PROMOTION AND CELL CULTURE STUDIES

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The carcinogenic process is multifactorial in its causation and multistage in its development. The two-stage model of chemical skin carcinogenesis is one of the best known experimental systems to study the mechanisms underlying the different steps in the cocarcinogenesis processes. Operationally two clearly separate events and mediated by different agents have been defined, namely tumor initiation and promotion. Tumor promoters are compounds which lack significant carcinogenic activity when tested alone, but markedly enhance the yield of tumors when applied after a low dose of an initiating carcinogen. The best known promoters are the phorbol-type diterpene esters and their promoting activity has also been shown in other organs than skin. Cell culture studies provided important clues to the unterstanding of tumor promotion and have revealed interesting biologic effects of the phorbol esters. These effects can be devided in four categories :

1) Mimicry of transformation in normal cells and enhancement of transformation by chemical carcinogens, viruses and X- or UV-rays,
2) Modulation (inhibition or enhancement) of differentiation and maturation,
3) Alteration of membranes resulting in changed receptor functions and intercellular communication,
4) gene modifications by chromatid and chromosomal changes.

Recent evidence suggests that phorbol esters bind to specific membrane receptors, lead to rapid alteration of membrane phospholipids and produce signals or mediators which lead to the subsequent cytoplasmic and nuclear effects. Although promoter effects can be observed with a variety of cell types, there is no unique alteration which is specific for phorbol-ester tumor promoters. The expression of the pleiotropic effects vary with tissue origin and functional state of cells and they were predominantly studied in mesenchyme-type cells. Although promotion related effects have been observed as well in epithelial cell cultures, they may vary qualitatively and quantitatively from those observed in fibroblast cultures. Stimulation of cell proliferation, inhibition of differentiation, enhancement of transformed phenotype and chromosomal modifications have been documented in epithelial cultures. Although promotion in vivo has so far nearly exclusively been demonstrated in epithelial tissues, two-stage transformation in vitro has only been demonstrated with mesenchymal cells. However, this has been predominantly realized with cells from permanent