non-carcinogenic agaritine was poorly converted. HMBD also reacted with adenine in vitro forming an unexpected adduct.

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ORAL TOBACCO USE AND THE POTENTIAL ENDOGENOUS FORMATION OF TOBACCO SPECIFIC NITROSAMINES UNDER SIMULATED GASTRIC CONDITIONS

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The exogenous exposure to tobacco specific nitrosamines (TSNAs), N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT), N-nitrosonornicotine (NNN) and 4-(methyl- nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in tobacco products is well documented. The potential endogenous formation of TSNAs from a variety of chewing tobaccos, oral snuffs, masheri and zarda samples was determined by extraction of tobacco samples with artificial saliva followed by incubation of extracts for 1 hr at 37° C and pH 2.0 under conditions simulating the normal fasting stomach with a constant 25µM nitrite concentration. Under the simulated gastric conditions, formation of NNN, NAB and NAT occurred. Nicotine, the major alkaloid present in tobacco and precursor to NNN and NNK was not nitrosated. The formation of NNN resulted from nitrosation of nornicotine, another alkaloid present in tobacco. Under the simulated gastric conditions, slight decomposition of NNK was observed.

The implications of the results from the model gastric nitrosations yielding NAB, NAT and NNN from various tobacco products and the additional potential exposure to TSNAs formed under in vivo conditions are being evaluated.

CYTOTOXICITY OF IL-2 ACTIVATED KILLER CELLS (LAK CELLS) AGAINST AUTOLOGOUS AND ALLOGENEIC INVASIVE BLADDER CANCER CELLS IN VITRO

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The ability of recombinant Interleukin-2 (RIL-2) activated peripheral blood cells (LAK cells) to kill autologous invasive bladder cancer cells and to kill cells from an established bladder cancer cell line, T24, was investigated.

Autologous tumour cell cultures were obtained from primary cultures derived from biopsies of transiotinal cell carcinomas. The outgrowing cells were harvested after 5 days of cultivation by trypsination (0.05%).

LAK cells were induced by incubating the peripheral blood cells (PBL) from the same patients with 50 Units/ml of RIL-2 for 3 to 6 days. PBL incubated without RIL-2 served as controls. The cytotoxic effect of the lymphocytes was evaluated by an 18 hr chromium release assay with a target:effector cell ratio of 1:50. The median tumour cell lysis induced with RIL-2 activatd PBLs was 20% for the autologous tumour cells and 35% for the T24 cells. The median lysis with the controls 4% and 6%.

EFFECTS OF NEIGHBOURING SEQUENCES ON THE SENSITIVITY OF GUANINE TO ALKYLATION LESIONS

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An important step in carcinogenesis is thought to be the initial attack on the DNA molecule by the ultimate carcinogen. An interesting class of carcinogens/mutagens are alkylating agents. It has been shown that alkylation of DNA and especially alkylation at the position O^6 of the guanine produces lesions that are associated with mutations (G- A) and neoplastic transformation. It was interesting to see if some guanines are more sensitive than others, to the mutagenic action of alkylating agents and the role of neighbouring sequences in the production of mutations.

In vitro alkylation was performed ona fragment of pBR 322 (fragment BamHI-SalI, 275 bp) containing the tetracycline resistance gene. The fragment was modified to various extents by MNU and was reinserted into the non-reacted large fragment. After transformation of E.Coli, mutants were selected for ampicillin resistance and tetracycline sensitivity. The mutants were analysed for sequence changes in the 275 bp fragment by the dideoxy method. Results, in terms of mutation distribution and neighbouring sequence effects, hae been obtained.

ESTRADIOL INDUCED PEROXIDASE ACTIVITY AS A MARKER OF HORMONE DEPENDENT HUMAN BREAST CANCER

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Study of estradiol receptor (ER) has been used as reliable tool for predicting results of hormonal treatment in human breast cancer. Approximately 40% of receptor positive patients did not respond favourably. In our study we used estradiol induced peroxidase (EC I.II.I.7) as another marker of hormone dependent breast cancer. In 96 primary breast carcinomas we determined ER and peroxidase activity after 24 hr stimulation in organ culture with 10^{-8}M estradiol. Peroxidase assays were performed by the rate of oxidation of guaiacol and were expressed as unit per mg protein (over 1 U/100 mg protein as positive). 33 carcinomas were ER and peroxidase positive and 32 of them responded favourably after endocrine treatment; 60 carcinomas were ER and peroxidase negative and only 3 of them responded. Estradiol induced peroxidase is therefore a potentially useful marker of hormone dependent human breast carinomas.

METASTATIC GROWTH OF HEPATOCARCINOMA CELLS IN F344 RATS AFTER SUBRENAL CAPSULE TRANSPLANTATION OF PRIMARY TUMOURS

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A metastasis-forming hepatocellular carcinoma was induced in a F344 male rat by a single injection of MNU of newborn age. The tumour was maintained by serial passage in F344 rats and metastases were found without exception on the peritoneum and in the parathymical lymph nodes. A new method was elaborated for standardization of tumour growth: transplant discs with equal size were prepared and put under the left kidney capsule. The progression of primary tumour tissue and its metastases could be followed by protein and DNA determinations. Enhanced tumour metastasis was observed after ablation of the left kidney even three days after tumour-transplantation. It is proposed that metastasis formation is a very early phenomenon, but metastatic cell growth is suppressed by primary tumour cells.

BENZO(A)PYRENE-DNA ADDUCTS - IMPLICATIONS OF EXPERIMENTAL AND HUMAN DATA FOR MONITORING

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Benzo(a)pyrene (BP) is an animal carcinogen, and it may contribute to lung cancer caused by cigarette smoke and occupational exposure to polycyclic aromatic hydrocarbons (PAH). We are using synchronous fluorescence spectrophotometry (SFS) and ultrasensitive enzymatic radioimmunoassay (USERIA) to measure BPDE-DNA-adducts, the putative carcinogenic lesion caused by BP-exposure. Although in controlled experimental situations, e.g. in animals treated in vivo with BP, both methods are very sensitive, quantitative and correlate well with each other, there are some unanswered questions as to the in vivo monitoring. The specificity of the methods is not complete when isolated PAH-DNA adducts are studied, but may still be adequate for in vivo monitoring. We have found some positive cases among human DNA isolated from blood cells of work-exposed or from placenta from smoking mothers. To further evaluate the contribution of cigarette smoking and the application of the methods we use, we are trying to set up an animal model. Although we have found a dose-dependent increase in the BPDE-DNA adduct formation in several organs after in vivo treatment of C57BL/6 and DBA/2 mice with BP, no adducts have been detected after cigarette smoke exposure in vivo, or injection of cigarette smoke condensate (CSC) or neutral fraction of CSC, even in the case where the AHH-activity was induced. It seems that in human tissues, similarly, there is a far-from-perfect correlation between the AHH-activity and in vivo or in vitro formed adducts.

MONO-OXYGENASE CATALYZED REACTIONS AND BINDING OF BENZO(A)PYRENE TO DNA IN HUMAN TISSUES. ROLE IN SUSCEPTIBILITY TO CHEMICALLY INDUCED CANCER

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We have studied whether the in vitro measured monooxygenase (MO) activities in human tissues are associated with susceptibility to chemical-induced cancers. Although activity and inducibility of various MOs show large inter-organ and inter-individual differences the induction of aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AHH) seems to be at least partially "systemically" regulated, thus