differences were seen in structure of c-Ha-ras proto-oncogene in the tumour and in the lymphocyte DNA isolated from the same person. However, some differences in several loci were detected when DNA containing multilocus repeated sequences (minisatellite DNA) was used as a probe. These results indicate that loss or redistribution of some DNA sequences occurs in neoplastic tissue.

THE SENSITIVITY TO CYTOSTATICS OF THE HUMAN MELANOMA XENOGRAFTS IN IMMUNE-DEPRIVED MICE

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We established two human malignant melanoma xenografts, MEL-1 and MEL-2, subcutaneously into immune-deprived mice by melanoma cells derived from the primary monolayer culture. The xenografts grew progressively till the animals death. Mel-1 was the faster growing tumour. Both xenografts spontaneously metastasized to the lung. The histological appearance of the xenografts and their metastases were similar to that of original tumours. Both melanoma xenografts were sensitive to DTIC and Cis-platinum in all parameters tested. It is concluded that the lung colony assay provides the best possibility to assess the antitumour activity of the cytostatics used.

ADDUCT FORMATION OF DIETHELSTILBESTROL AND STEROIDAL ESTROGENS WITH AMINO ACIDS IN VITRO

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Recent evidence suggests that the induction of changes in chromosome number, i.e. aneuploidy, is a critical event in the process of neoplastic cell transformation induced by stilbene estrogens such as diethylstilbestrol (DES) and also by natural estrogens and their metabolites. We postulate that the biochemical mechanism underlying aneuploidy induction by estrogens involves covalent binding of metabolically activated estrogens to proteins of the spindle apparatus. In support of this proposition, we have recently demonstrated that DES and 2-hydroxy estradiol (2-HO-E2), the major metabolite of estradiol-17β, are able to bind covalently to a specific binding site in the C-terminal region of tubulin upon activation with peroxidase/hydrogen peroxide. In order to identify the binding amino acid(s), we have now studied the reactivity of perioxidative metabolites of DES and 2-HO-E_2 towards various amino acids in vitro. Using [14]C-labelled estrogens and high performance liquid chromatography, we found the greatest extent of adduct formation with cysteine and tyrosine, while other amino acids gave only small amounts of adducts or did not react at all. This preferential binding of reactive estrogen metabolites to certain amino acids may help to explain the observed specificity in the covalent binding to tubulin.

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MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS AND THE EFFECT ON SOME MARKER ENZYMES BY PEROXISOME PROLIFERATORS

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Many hypolipidemic drugs and industrial plasticizers cause peroxisome proliferation in rat liver, and induce hepatic neoplasms in rats and mice. The peroxisome proliferators (PPs) show little or no evidence of direct interaction with DNA. The effects of the PPs clofibrate (CLO) and diethylhexyl phthalate (DEHP) on different marker enzymes and of morphological transformation are studied in Syrian hamster embryo (SHE) cells. Preliminary results indicate that both chemicals induce morphological transformation of SHE cells. They induced an increase in catalase activity (peroxisomes), while no increase was found for glucose-6-phosphatase (endoplasmic reticulum) and acid phosphatase (lysosomes). Malate dehydrogenase (mitochondria) showed more inconsistent results. We were not able to detect peroxisomal beta-oxidation in either PP-exposed or control cells. Electron microscopical studies of SHE cell peroxisomes are in progress.

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COMPARISON OF THE ABILITY OF GLASS FIBERS AND ASBESTOS TO INDUCE MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS

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The ability of glass fibers and different types of asbestos to induce morphological transformation of Syrian hamster embryo cells has been compared. An increased transformation frequency was obtained with glass fiber (GF) 100 as well as chrysolite, crocidolite, amosite and anthophyllite, while no significant increase was observed for GF 100 and TiO_2. GF 100 was less potent than chrysolite, but more potent than crocidolite, amosite and anthophyllite. By comparing the transformation frequency and toxicity, it could be concluded that induction of transformation could not be caused by unspecific cytotoxic effects. In contrast to earlier studies, no synergistic effect was observed between benzo(a)pyrene and asbestos fibers. Electron microscopical studies show that the fibers were rapidly phagocytosed, and blebs appeared on the cell surface. The formation of blebs depended on the fibers used. The localization of the blebs seemed not to be specifically associated with area of physical interaction with the fibers.

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CYTOTOXICITY OF BENFLURON METABOLITES ON P388 AND EHRLICH CELLS

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The new cytostatic drug Benfluron is currently being tested in clinical trials. In the present study the cytotoxicities and mode of action of 2 metabolites of Benfluron(A), namely 7-dihydrobenfluron(B) and N-oxide(C), have been investigated. Both metabolites are cytotoxic against the two tumour types tested (P388 and Ehrlich tumour cells) but they are less active than the parent compound (A). In order to elucidate the mode of action, the effects of both B and C on aerobic glycolysis, different kinds of respiration, level of ATP and thiol groups, integrity of cell membranes and loss of transplantability have been compared. Both B and C have shown at least two modes of action according to the concentration tested. In low concentration both metabolites interfere probably with DNA synthesis and subsequently with RNA and protein biosynthesis. At the highest concentrations there is damage to cell membranes.

DNA DAMAGE AND IN VITRO EVALUATION OF ANTICANCER DRUG SCHEDULE DEPENDENCY OF N,N-BIS(CHLORETHYL)-3-CHLORPROPIOAMIDINE OXALATE

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It has been recently shown that N,N-bis(chlorethyl)-3-chlorpropioamidine oxalate possesses antitumour activity in vivo and is worthy of further evaluation. This study was aimed at characterization of its cytotoxicity in vitro and elucidating the possible mechanism of action in terms of effects on cellular DNA. As a result it was found (by clonogenic cell survival assay) that the drug produced exponential reduction in cell survival and similarly shaped dose-response curves when given by short or continuous exposure. That data, as well as the low ID50 ratio, characterize the cytotoxicity of the drug as schedule independent and suggest cell cycle non-specificity. It was found also (by using DNA unwinding assay and a nucleoid sedimentation technique) that the drug in pharmacologically relevant concentrations caused single DNA strand breaks, which increased with drug concentration. Considering all the data, it is probable that drug-induced DNA breaks are the major cause for the drug cytotoxicity.

PROGRESSION OF ENDOMETRIAL ADENOCARCINOMAS AS REFLECTED BY NUCLEAR DNA CONTENT AND CELLULAR ESTROGEN RECEPTORS

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The results from a combined retro- and prospective study of variations of nuclear DNA and cellular estrogen receptors of normal and hyperblastic endometrium and of endometrial adenocarcinomas in relation to the clinical stage, the histological grade and the growth pattern of the tumours, to the incidence of metastases and to the survival rates of the patients has been evaluated. The errors and the validity of DNA measurements on smear preparations and histological sections by direct microspectrophotometry and of flow cytometric determination of tumour cell