Human melanoma variants and clones with increased metastatic abilities were obtained from melanoma cell lines in nude mice and in immunosuppressed new-born rats. Subcutaneous transplantation in a nude mouse of a human melanoma metastatic nodule resulted in a subcutaneous tumour (NTT) and in spontaneous lung (NTP) and lymph node (NTG) metastases (Neulat-Duga et al., Invasion and Metastasis, 4: 209-224, 1984) which were first maintained in vivo by subcutaneous passages in nude mice and then cultured in vitro as cell lines. Cytogenetic studies showed that all three tumour lines have a common origin and that metastases resulted from a population selection. After 15 in vitro passages, NTP cells were injected s.c. in nude mice : serial transplantation was accompanied by an increase in metastatic abilities of tumour cells. Melanoma cell lines, tumourigenic but non metastatic in nude mice, were xenografted to ATS-treated new-born rats. 3 weeks after s.c. injection of 10^6 cells, nearly all rats developed tumours and a proportion of them had lung and lymph node metastases. Agar cloning of M4Beu line showed that it is heterogeneous and contains poorly tumourigenic, but highly metastatic cells.

DETECTION OF AFLATOXIN-LIKE SUBSTANCES IN THE GENERAL DANISH POPULATION

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A competitive ELISA assay for detection of Aflatoxin B1 (AFB) in urine has been developed using a monoclonal anti-AFB antibody. The assay has been characterized with respect to sensitivity towards a range of aflatoxins and derivatives. The aromatic structures of AFB with the anisole group as well as the lactone region are required for competitive action in the assay. AFB concentrations down to 0.1 ng/ml could be detected.

Most urine samples from 80 normal Danish volunteers were positive in this assay, containing 0.1 to 10 ng-eqv. AFB per ml. The structure of the urinary aflatoxin-like antigenic substance (ALAS) is presently unknown. ALAS is a true competitor with AFB for the antibody and can be concentrated by affinity chromatography. We are presently attempting to identify the chemical structure of ALAS.

LOSS OF HETEROZYGOSITY ON CHROMOSOME 22 IN PRIMARY TUMOUR MATERIAL FROM MENINGIOMA

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Cytogenetic analyses have shown that monosomy 22 is common in primary cultures of meningioma. A small fraction of these tumours have also shown deletions on chromosome 22. We have analysed restriction fragment length alleles at eleven loci on chromosome 22 in primary tumour material and the corresponding constitutional tissue from 20 patients with meningiomas, using polymorphic DNA markers. Loss of constitutional alleles along the whole chromosome 22 were found in 6 cases, consistent with a non-disjunction event. In addition, 5 meningiomas showed loss of alleles on part of chromosome 22 while at least one other locus on chromosome 22 showed retained heterozygosity. Analysis of polymorphic loci on 15 other chromosomes revealed only a few tumours with single losses. A more extensive analysis of cases with deletions may help to localise a recessive meningioma gene regionally on chromosome 22.

DNA DAMAGE AND THIOL DEPLETION CAUSED BY FECAPENTAENE-12 IN HUMAN FIBROBLASTS

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Fecapentaene-12 (fec-12) is a fecal mutagen that also is genotoxic in cultured human fibroblasts (Plummer et al, Carcinogenesis 7, 1607-1609, 1986). Further studies indicate that survival of fibroblasts measured as colony forming efficiency or trypan blue exclusion was decreased to approximately 50% between 0.5 to 1.0 µm fec-12 after either 1, 3 or 24 hr exposure times. The cellular content of total thiols (mainly glutathione) was decreased in a dose dependent manner up to 1.0 µM fec-12 which decreased thiol content to 60% of control. Higher doses of fec-12 did not cause further thiol depletion. Because depletion of GSH was not coupled with formation of GSSG, these results indicate that fec-12 depletes cellular thiols by direct conjugation. As analyzed by alkaline elution, fec-12 also caused several types of DNA damage. Primarily DNA

interstrand crosslinks were detected at 2 μM fec-12. Higher doses of fec-12 also caused DNA single strand breaks, DNA protein crosslinks and alkali-labile sites. Dose-effect studies indicate both the high reactivity of fec-12 towards cellular thiols and DNA, and that cellular thiols are of crucial importance in protecting human cells against fec-12-induced DNA damage.

CHANGES IN GLYCOSAMINOGLYCAN SYNTHESIS AND CELLULAR ADHESION ASSOCIATED WITH INCREASSED METASTATIC POTENTIAL OF MELANOMA CELLS

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In order to determine if compositional or structural changes of glycosaminoglycans (GAGS) are associated with the metastic efficiency of tumour cells, we have examined the composition of GAGS, and the properties of heparans synthesised by B16F1, F10 and BL6 melanoma cells, in addition to cellular adhesion studies.

The poorly metatatic cell line B16F1 synthesised a higher proportion of non-sulphated GAGS compared to those synthesised by the metastatic F10 and BL6 cell lines. However, there was no significant difference in the composition of the sulphated GAGS between the three cell lines. Heparans from the metastatic F10 and BL6 cells bound more tightly to DEAE-cellulose columns than the heparans isolated from the F1 cells. The metastatic cells synthesised heparans of a higher molecular weight, while there was no detectable difference in the degree of sulphation. The B16F1 cells exhibited only poor cell-cell adhesion when seeded on agar compared to the F10 and B16 cells, while all three cell lines eventually formed spheroid-like structures when seeded on basement membrane gel. The observed changes in the GAGS may contribute to the arrest or attachment of tumour cells to the endothelium of the target organ.

CARCINOGENICITY AND MUTAGENICITY STUDIES IN NEW DRUG APPLICATIONS

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The Nordic guidelines on new drug applications specify a detailed list of mutagenicity and carcinogenicity data required for the registration of drugs intended for chronic use, e.g. non-steroidal anti-inflammatory drugs (NSAIDs). I have analysed the data available to the Finnish licencing authority on NSAID applications submitted during 1976-83. The criteria used were quantitative, so the technical and scientific aspects of the test procedures did not fall within the scope of the study.

Mutagenicity studies were included in 33% of applications according to the guidelines, 33% were inadequate and 33% of applications did not contain any data on genotoxicology at all. Carcinogenicity studies were reported adequately in 39% of applications, but 39% were inadequate and 22% of applications failed to document any carcinogenicity studies.

The analysis of mutagenicity and carcinogenicity data revealed clear defects. Inadequacies in documentation lead to enquiry of additional data which prolongs registration times. However, the proportion of adequate applications increased as a function of time during 1976-83.

CELL PHENOTYPE INSTABILITY IN PRENEOPLASTIC FOCI OF RAT LIVER

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Carcinogenesis is a multi-step process in which genetic-phenotypic instability and sequential selection of preneoplastic cells for increased growth capacity and other neoplastic characteristics are essential phenomena. During chemical carcinogenesis in rat liver, the development of enzyme defficient foci, their clonal origin and their relationship to tumour formation are known. We report the results of four carcinogenesis protocols consisting in one or two cycles of diethylnitrosamine and phenobarbital. Histochemistry for three enzymes on serial sections has revealed seven different kinds of homogeneous liver foci resulting from simple and combined enzyme deficiencies and also heterogeneous foci showing small foci inside. We consider such secondary foci as subclones originated from cells already modified that have developed an additional phenotypic change. Some of such foci develop after the first cycle if the promotion phase is as long as 57 weeks but their appearance is much more important after a second cycle. Comparing the number of foci per surface area of liver section with the number of secondary foci per surface area of focus section, it seems clear that cells already modified are less stable than other hepatocytes, showing a