A EUKARYOTIC TEST FOR CARCINOGENS BASED ON DNA MODIFICATION, TRANSFER TO LTK CELLS AND EXPRESSION STUDIES. M.Schaefer-Ridder, T.Mörðy and U.Engelhardt. Max Planck Institut für Biochemie, 8033 Martinsried, F.R.G.

The ultimate carcinogenic metabolites of benzo(a)pyrene, benzo(a)pyrene-7, 8-dihydrodiol-9, 10-epoxide and several other chemicals were reacted in vitro with a plasmid containing the thymidine kinase gene. The electrophoretic mobility of the supercoiled DNA was changed beyond a critical dosage level of carcinogen and the covalently modified DNA was no longer recognized by restriction endonucleases. The modification pattern was quantitatively correlated to the biological activity by transfer of the TK gene - modified and unmodified - to TK deficient cells. Upon transfection of mouse LTK$^-$ cells with the modified gene (plasmid) no TK$^+$ cells were obtained in contrast to the formation of many colonies after transfection with the unmodified gene. The carcinogenic activity of the chemicals could be evaluated by comparing the number of colonies at a certain dosage of chemicals. There was a clear difference between strong, weak and inactive carcinogens. Gene inactivation was also observed for another DNA, the gene of Hepatitis B virus surface antigen.

THE PUTATIVE SECOND-STAGE LESION IN RAT LIVER CARCINOGENESIS. E.Scherer, A.W.Feringa and P.Emmelot. Division of Chemical Carcinogenesis, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands.

Whereas the first cell stage of rat liver carcinogenesis, the enzyme-altered island, is well studied little is known about the further development into malignant liver cancer. To overcome this, an experimental protocol has been developed for the synchronized induction of a second-stage lesion. This protocol sequentially combines an initiating dose of carcinogen (DEN), a strong proliferative stimulus for initiated cells and a second dose of a carcinogen (ENU, DEN). It leads to the formation of new foci within the confines of islands. These foci have already characteristics of hepatocellular carcinoma, e.g. evenly distributed (slight) cytoplasmic basophilia, some loss of glycogen storage indicating a further change of carbohydrate metabolism and slow proliferation to macroscopic size (up to 2 cm within 190 days). The probability for the change island → tumour cell is in the same order of magnitude as that for the change liver cell → island cell (same level of DNA ethylation) indicating that in both steps similar genetic events are involved. The possible importance of such rare events during the promotion phase of carcinogenesis is stressed.

NEONATAL MORTALITY RATES AS A POSSIBLE RISK ASSESSMENT FACTOR IN EXPERIMENTAL TRANSPLACENTAL CARCINOGENESIS. Wolfgang Schmahl, Abteilung für Nuklearbiologie der G.S.F., München, F.R.G.

5-Azacytidine is an effective transplacental carcinogen in NMRImice, especially when administered on day 12 or on day 16 of gestation. It evokes mainly lymphoblastic leukaemias and lung and liver adenomas. Tumour incidences are, however, not uniformly distributed between all offspring, but cumulate in different litters. We looked for a possible correlation between the neonatal mortality rates in individual litters (as a parameter of embryo toxicity effects) and their later tumour frequencies. Control animals displayed a homogeneous distribution of carcinogenic effects within the whole scale of mortality rates. Azacytidine application on day 12, however, resulted in a significantly lower tumour incidence in those litters suffering from a neonatal mortality rate lower than 15%, than in the higher mortality rate - affected litters. In the latter, this effect is largely due to a twofold increase of leukaemia incidence (29.7% to 59.8%) and of lung tumour incidence (14.1% to 27.4%). However, liver tumour incidence is about twice as high in the low mortality-litters (7.5%) than in the high mortality-litters.