CHANGES IN ENZYMATIC DNA METHYLATION CAUSED BY CHEMICAL CARCINOGENS
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Experiments were performed in order to elucidate the possible role of an altered process of enzymatic methylation of DNA cytosines in the initiation of chemical carcinogenesis. Cells in tissue culture were treated with L-ethionine, N-methyl-N-nitrosourea, and N-acetoxy-N-2-acetylaminofluorene, and the extent of enzymatic methylation of DNA cytosines was measured before, during, and after treatment with these carcinogens. The results with precursor incorporation studies and restriction enzyme analyses suggest that these carcinogens alter the extent of enzymatic methylation of DNA cytosines. This is accompanied by changes in the transcriptional complexity in treated cells. The aberrant methylation patterns persist in the absence of the carcinogens. Cellular cloning experiments show that the carcinogens also induce cellular heterogeneity in a previously homogeneous cell population with respect to enzymatic DNA methylation. Thus, the altered DNA methylation can stably fix a carcinogen-damage to the cell.

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NUCLEAR PROTEIN DAMAGE BY ALKYLATING AGENTS WITH DIFFERENT DEGREES OF CARCINOGENIC AND MUTAGENIC POTENCY.
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We have attempted to establish if there is any correlation between the carcinogenic and mutagenic potency of alkylating compounds and specific target sites in chromatin, other than a high ratio O^6/N^7-methyl-guanine in DNA. We have therefore analyzed the nuclear metabolism of radioactively labelled alkylating agents in cultured cells with (primary hepatocytes) or without (V79) microsomal activity. We have studied so far: 1)methylmethanesulphonate (MMS), a relatively weak carcinogen and Ingold $S_N2$alkylating agent, 2)N-methyl-nitrosourea (MNUA), a highly potent carcinogen close to a typical Ingold S_N1. Cells in culture were exposed to different doses of alkylating agents for 18 hr . Histones (H) and non-histone-nuclear proteins (NHNP) were extracted and then hydrolyzed in the presence of β-mercaptoethanol. Hydrolyzates were eluted with HCl from a Dowex 50Wx8 column, where all methylated amino acids can be separated. We found that, at all doses, MMS methylates H and NHNP on Cysteine (Cys) and to a lower extent Histidine (His). Traces of methylated Arginine(Arg) and Lysine(Lys) can be detected(higher in presence of microsomal activity). MNUA at low dosage methylates only Lys and Arg: met-Lys representing the largest percentage of total counts in H, met-Arg in NHNP. Methylated amino acids account for a higher percentage of total radioactivity incorporated into H and NHNP, in hepatocytes than in V79. Traces of met-Cys and met-His can be detected at high MNUA dosage. A new specificity is suggested.

DNA DAMAGE INDUCED BY BENZO(a)PYRENE IN MOUSE FOETAL TISSUES.
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We are investigating the possible relationship between organotropism displayed by many chemical carcinogens and the amount and persistence of DNA damage in target tissues of foetal mice. As a first approach 15-day old Swiss mouse foetuses were exposed to 10 mg/Kg body weight of benzo(a)pyrene (BP) by maternal or intrafoetal injections. Control animals received trioctanoin and acetone (1:1). Females were sacrificed at 4,24,48 and 72 hr after treatment and nuclei isolated from foetal pulmonary and hepatic cells. DNA damage was evaluated utilizing the alkaline elution technique and a microfluorimetric DNA determination. There were 3 litters per time point. In BP treated foetuses the alkaline elution constants (K) peaked at 4 hr and were 0.052 and 0.071 for the liver and 0.052 and 0.044 for lung following maternal or intrafoetal treatments, respectively (controls were 0.019 and 0.027 respectively). In both organs K remained roughly constant until 48 hr and at 72 hr the amount of DNA damage returned to the control level. These results indicate that foetal liver is already capable of metabolizing BP to toxic derivatives. Since the lung but not the liver is a target organ for BP carcinogenesis, they further suggest an absence of correlation between the induced DNA damage and the insurgence of tumours. It must be noted, however, that the liver has different functions in prenatal as compared to postnatal life and this phenomenon may be relevant in explaining our results.