SHORT COMMUNICATION

\textbf{32P-Postlabeling analysis of liver DNA adducts in rats chronically fed a choline-devoid diet}

Ramesh C. Gupta\textsuperscript{1}, Karen Earley\textsuperscript{1}, Joseph Locker\textsuperscript{2} and Benito Lombardi\textsuperscript{2,3}

\textsuperscript{1}Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030, and \textsuperscript{2}Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261, USA

\textsuperscript{*To whom correspondence should be sent

Liver DNA, obtained at various time intervals from rats chronically fed a choline-devoid diet, was analysed for the presence of aromatic or alkyl adducts by the \textsuperscript{32P}-postlabeling assay. Alkyl adducts were not detected. Aromatic DNA adduct lesions were revealed, but only at levels (1 adduct per $0.5-3 \times 10^9$ nucleotides) which are at the limits of the extremely high sensitivity of the method used, levels which remained constant throughout the period of feeding. Thus, contamination of the total environment of the animals with chemical carcinogens does not appear to be responsible for the genesis of the hepatocellular carcinomas that develop in rats chronically fed a choline-devoid diet. The diet, therefore, either acts as a complete carcinogen, or promotes the evolution to cancer of endogenous, 'spontaneously' initiated liver cells.

Recent findings (1-3) have shown that a high incidence of metastatic hepatocellular carcinomas (HCCs)\textsuperscript{*} develops in rats chronically fed a choline-devoid (CD) diet and not exposed intentionally to chemical carcinogens. In contrast to what might have been the case in earlier studies (4,5), the diets were found to contain no significant level of likely chemical carcinogen contaminants, including aflatoxins, leading to the conclusion that a CD diet is a complete carcinogen, able to initiate liver cells, as well as to promote the evolution of initiated cells to HCCs (1-5). However, the possibility remained that other relevant chemical contaminant(s) in the diet might have been overlooked, or might have been present in the drinking water or the ambient environment of the animals. To assess this possibility, liver DNA adduct analyses were performed by means of the \textsuperscript{32P}-postlabeling assay (6,7). This method has an extremely high sensitivity, since it can detect one modified residue, generated by aromatic or alkylating hydrocarbon-DNA adduct lesions, revealed, but only at levels (1 adduct per $0.5-3 \times 10^9$ nucleotides) which are at the limits of the extremely high sensitivity of the method used, levels which remained constant throughout the period of feeding. Thus, contamination of the total environment of the animals with chemical carcinogens does not appear to be responsible for the genesis of the hepatocellular carcinomas that develop in rats chronically fed a choline-devoid diet. The diet, therefore, either acts as a complete carcinogen, or promotes the evolution to cancer of endogenous, 'spontaneously' initiated liver cells.

\textsuperscript{*Abbreviations: HCCs, hepatocellular carcinomas; CD, choline-devoid; CS, choline-supplemented; AAP, 2-acetylaminophenanthrene; B[a]P, benzo[a]pyrene.}

© IRL Press Limited, Oxford, England

\textsuperscript{© IRL Press Limited, Oxford, England}
adducts. For this purpose, liver DNA was analyzed for the presence of aromatic or alkyl adducts by means of the \(^{32}\)P-postlabeling assay (6–8). This method has an extremely high sensitivity, even though identification of adducts depends principally on availability of relative reference compounds. The presence of alkyl adducts was not revealed by the analyses, while identical aromatic DNA adduct lesions were detected in liver DNA of rats fed the CD diet, or a control CS diet, but only at the limits of sensitivity of the method, and at invariable concentrations during the whole period of experimentation. The results, therefore, do not seem to support the possibility that conspicuous environmental contaminant(s) are responsible for the genesis of HCCs in rats fed a CD diet, and seem to lend instead weight to the conclusion that the diet acts as a complete carcinogen (1–5). However, there is increasing evidence (see 13) that the liver of several strains of rats, including Fisher-344 rats, contains cells capable of evolution toward cancer, due to 'endogenous' initiation by relevant 'background' factors and agents (14). One wonders, therefore, whether the barely detectable concentrations of adducts, observed in the present study, might not be related to, or an index of, the presence of endogenous initiated cells in the rats used in these experiments, a possibility clearly suggested also by findings reported by Randerath et al. (15) after submission of this paper. Were this the case, it could be argued that initiated liver cells were present in rats fed both the CD and the CS diet, but that only in rats fed the CD diet did the cells evolve to HCCs, because of the promoting action of the diet (16, 17).

Acknowledgements
The Authors thank L.Larson for technical assistance and M.L.Rotz for manuscript preparation. This work was supported in part by grants from the National Institutes of Health (CA23449 and CA30606); the American Cancer Society (BC471); and the Council for Tobacco Research (1634).

References


Received on 18 July 1986; accepted on 2 October 1986