Journal of Environment & Pollution 3(1), 1996 pp. 45-47
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CERTAIN ASPECTS OF HISTOLOGICAL CHANGES IN INDUCED HEPATOCARCINOGENESIS BY DIETHYLNITROSAMINE

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ABSTRACT

After 30 days of introduction (200 mg/kg body weight) of diethylnitrosamine (DEN) - a potent carcinogen, the Weister albino rats were killed and the study of the histological preparation of their liver revealed appearance of foci, cells with vesicular nucleus, clumping of basophilic cells and proliferation of only altered hepatocytes. The details of histological changes of this stage of hepatocarcinogenesis has been discussed.

INTRODUCTION

Tumours induced *in vivo* in animals are predominantly carcinomas and are tissue specific (Iype et al. 1975). Right from exposure of the carcinogen to the ultimate manifestation - the formation of tumours - the target organs undergo a number of changes (Holley 1972, Craddock 1973, Solt and Farber 1976, Cayama et al. 1978). During inoculation, DNA becomes the primary target of chemical carcinogens (Fialkov 1972) as the carcinogens become associated with nucleic acid in the respective animal tissue either as a consequence of direct chemical reaction or following their metabolic conversion to relative metabolites (Irving 1973). The induction of cancer by chemicals appear to be a two-stage process. The first stage involves reaction with cell constituents followed by prolonged second phase before the eventual appearance of tumour. Since hepatic cells are involved in metabolism of various distal chemical carcinogens (Iype et al. 1975) and have high potentiality of regeneration (Sinha 1983, Sinha and Sinha 1987), we have used liver of Weister albino rats for our investigation that has been directed to find out the sequential histological changes during induced carcinogenesis.

MATERIAL AND METHODS

Weister albino rats were used for the experiments because they appreciably withstand surgical procedures and have low postoperative mortality. The rats were obtained from Zoological Animal Emporium, Varanasi and were fed with the feed manufactured by Hindustan Lever Ltd., Bombay. The carcinogen was obtained from Paterson Laboratory, Christie Hospital and Holf Radium Institute, Manchester, England. Partial hepatectomy (PH) was done according to the methods described by Higgins and Anderson (1931).

To study the chemical hepatocarcinogenesis in the present study, a new assay system developed by Solt and Farber (1976), was employed which comprised of three components, a potent growth stimulus of hepatocytes - here through partial hepatectomy (PH), an initiator - the

carcinogen nitroso compound (DEN), and a selective growth inhibitor-2 Acetylaminofluorene (2AAF)

In PH about 67% of liver was removed in order to allow a rapid regeneration of the hepatocytes thereby allowing rapid turnover of DNA in multiplying cells. The carcinogen (DEN) has been administered after 20-22 hours of PH because at this stage maximum DNA synthesis takes place enabling the possible interaction necessary to bring about the gene mutation and expression. With such alteration, DNA in the dividing cells - altered hepatocytes, can be perpetuated through subsequent generations. The selective growth inhibitor in the form of 2AAF had been employed after one week of the administration of carcinogen because during seven days the inoculated carcinogen reacted completely with the hepatocytes and produced certain mutational changes at nucleic acid level, which ultimately produced the altered or deviated hepatocytes. The application of 2AAF inhibited the further proliferation of normal hepatocytes due to their resistance to 2AAF. Such manipulations enabled to study the sequential changes in the hepatocytes treated with carcinogen following PH.

RESULTS

The rats were killed on 30th day of carcinogen introduction to study the histological changes after complete regeneration of liver. The microscopic examination of the histological preparation revealed most significant observation that the appearance of various foci. The foci represent the area of basophilic cells (Fig. 1). The basophilic cells formed thickened clumps and exhibited active mitosis with occasional abnormal metaphase configuration (Fig. 2). Enlarged and irregular nuclei were frequently evident. There was a general restitution of the normal acinar architecture. Proliferation of a few oval cells took place in the portal area and in the periportal zones. Ongoing necrosis was not seen. Rare nuclear pleomorphism was demonstrated in the hepatocytes with frequent basophilic clumping of the hepatocytes cytoplasm. The characteristic feature of the cells of foci was vesicular nucleus with prominent nucleolus. There was no evidence of hepatocyte proliferation of parenchyma. Some occasional foci of altered hepatocytes were observed.

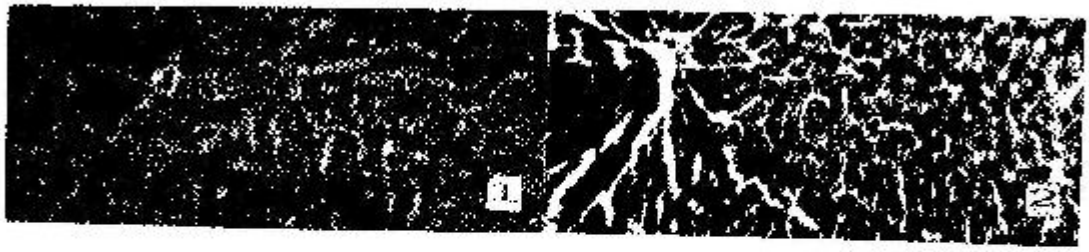

Fig. 1 The foci representing the area of basophilic cells. (X 150 H & E).
Fig. 2 The basophilic cells formed thickened clumps showing abnormality. (X 300 H & E).

DISCUSSION

Appearance of foci represented area of basophilic cells which formed thickened clumps and exhibited active mitosis with occasional abnormal metaphase configuration. Enlarged and irregular nuclei were frequently evident. The origin of these basophilic cells of the foci has been explained by Solt and Farber (1976, 1977). Initial stages of cell division showed mispairing of

alkylated base during DNA replication, thereby converting the transitory abnormality of aberrant methylation into an inheritable change in nucleotide base sequence (Craddock 1973). The innoculation of selective growth inhibitor (2AAF), made proliferation of normal or unaltered hepatocytes to be inhibited, whereas it did not inhibit the proliferation of the altered resistant hepatocytes (Solt et al. 1980). The altered hepatocytes resistant to 2AAF underwent massive proliferation as the result of which, foci of the altered resistant hepatocytes developed with gradual increase in size. The basophilic cells formed thickened clumps and exhibited active mitosis. Enlarged and irregular nuclei were frequently evident. The nuclei underwent profound modification and become enlarged and irregular in basophilic cells or altered hepatocytes were vesicular with prominent nucleoli, general restitution of the normal acinar architecture were seen due to the regeneration of liver has been completed at about 30th day (Sinha 1983).

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CONFERENCES / SYMPOSIA

INTECOL's V International Wetlands Conference. Perth, W. Australia, 22-28 September 1996. Contact : Secretariat, UWA Extension Conference Manager, University of Western Australia, Nedlands, Perth 6907, Australia.

Regional Seminar on Forests of the Humid Tropics of South and South East Asia. Kandy, Sri Lanka, 19-22 March 1996. Contact : Dr. P. Zoysa, MAB National Committee, Natural Resources, Energy and Science Authority, 47/5 Maitland Place, Colombo 7, Sri Lanka.

Third International Conference on Environmental Planning and Management. February 24-26, 1996. Visvesvaraya Regional College of Engineering, Nagpur, Maharashtra. Contact : Dr. A.G. Bhole, Convener ICEPM-66, HOD, Civil Engg. VRCE, Nagpur-440 011, Maharashtra, India.

22nd WEDC Conference and 19th National Convention of IPHE, India, Sept. 9-13, 1996. Organized jointly by Water, Engineering and Development Centre, Loughborough University of Technology, England and the Institute of Public Health Engineers, India at New Delhi, India. The papers can be sent to Prof. K.J. Nath (Chairman) or Mr. S.K. Neogi (Organizing Secretary), IPHE Building, CK-58, Salt Lake, Calcutta-700 091.

International Symposium on Infrastructure of the Future, November 25-29, 1996 at Bangalore. Themes are Power, Transportation, Telecommunication, Environment (related to power, transportation and telecommunication). For details contact : Secretary, ISF-96, Association of Consulting Civil Engineers (India), No.2, UVCF Alumni Association Building, K.R. Circle, Bangalore-560, India.

International Conference on Environmental Biotechnology, September 1-4, 1996 at Massey University, Palmerston North, New Zealand. For details contact : Conference Secretary, Environmental Biotechnology Conference, Process & Environmental Technology Deptt., Massey University, Palmerston North, New Zealand.

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