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HISTOLOGICAL CHANGES DURING HEPATOCARCINOGENESIS AFTER EXPOSURE OF DIETHYLNITROSAMINE IN WISTER ALBINO RATS

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ABSTRACT

At a dose level of 200 mg/kg body weight of a potent carcinogen diethylnitrosamine (CEN) which also occur in environment and could be formed in the body, was given to partially hepatectomised wister albino rats by intramuscular injection to Induce hepatocarcinogenesis. Two weeks after carcinogen introduction rats were killed and histological slides of liver was prepared to study histological changes in hepatocarcinogenesis. The study revealed formation of foci which are ATpase deficient group of cell and proliferative growth of oval basophilic hepatocytes with pleomorphism in nuclei.

INTRODUCTION

Human race is very much afraid of one single disease the cancer which in the present decade is still a major cause of human suffering and death. Cancer is a disease of multifactorial etiology to which envirnmental factors such as some carcinogenic pollutants, occupational exposures, smoking etc. in addition to life style factors contribute importantly, sometimes interactively (Cairns 1975).

Development in the past two decades have led to progressive shift in emphasis from individual to the environmental factors particularly pollutants in the perception of the causation and pathogenesis of cancer (Cairns 1981). An increasing number of dietary and environmental components including pesticides, fungicided and others widely used in food production are now being recognized as agents of cancer (Sihna 1983).

Laquer (1964) Laquer and Matsumote (1966) and Miller (1970) reported that exposure to certain chemicales played a significant role in causation of some neoplastic diseases in animals including man. But due to lack of scientific base it was not known as to what percentage of human cancer was particularly due to chemiclas. Frequently now it has been suggested that more than 80 percent of human cancers are developed due to environmental factors (Cairns 1981). This view in recent years has served an

useful purpose by emphasising the need for research into caucinogenic hazards from environmental chemicals.

The present paper deals with the initial histological changes occuring in the hepatocytes and liver as a whole of wister albino rats in vivo after intramuscular introduction of known chemical carcinogens Diethy-Initrosamine (DEN) - a nitrosocompound also occur in environment (Magee 1971, 1972, Wolff and Wasserman, 1972) and could be formed in the body itself (Lijinsky et. al. 1972) in order to get a better understanding of chemical carcinogenesis.

MATERIALS AND METHODS

For experimental purpose Wister albino rates were used because they appreciably withstand surgical procedures and post operative mortality among them usually low. The rates were obtained from Zoological Animal Emporium, Varanasi and were fed the feed manufactured by Hindustan Liver Ltd., Bombay. The carcinogen was obtained from Paterson Laboratory, Christiam Hospital and Holf Radium Institute, Manchester, England, Partial hepatectomy (PH) was done according to the methods described by Higgins and Andrson (1931)

To study the chemical hepatocarcinogenesis in the present studies a new essay system developed by Dennis (1976) was employed which comprised of three components, a potent growth stimulus of hepatocytes here through partial hepatectomy (PH), an initiator the cacrinogen nitrosocompound (DEN) and a selective growth inhibitor-2 Acetylaminofluorene (2AAF).

In PH about 67 percent of liver was removed in order to allow a rapid regeneration of the hepatocytes there by allowing rapid turn over of DNA in multiplying cells. The carcinogen (DEN) had been administrated 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 dwindeing cells altered hepatocytes, can be perpetuated through subsequent generations. The selective growth inhibitor in the form of 2 AAF had been employed after one week of the administration of carcinogen because during

Fig. 1 : Appearance of foci showing initial stage of carcinoma. (H & E) X 100.

seven days the inoculated carcinogen reacted completely with the hepatocytes and produced certain mutation changes at nucleic acid level, which ultimately produced the altered or deviated hepatocytes. The application of 2 AFF inhibited the further proiferation of normal hepatocytes while still allowing the proliferative growth of the altered hepatocytes, due to being resistant to the selective inhibitor (2 AAF). Such manipulations enabled to study the sequential changes in the hepatocytes treated with carcinogen following PH.

RESULTS

The complete regeneration period of

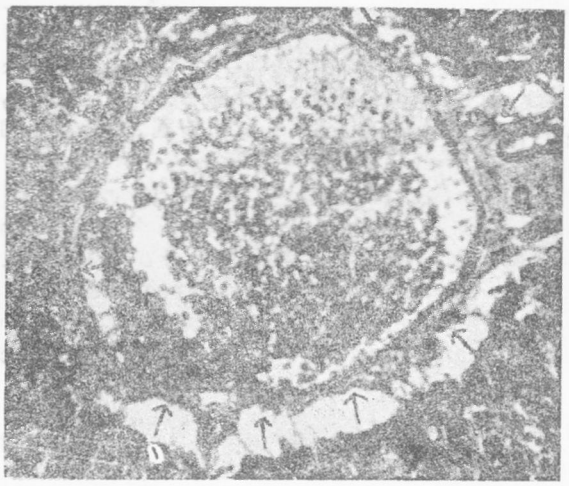

Fig. 2 : Necrotic hepatocytes in the area of terminal hepatic venules (H & E) X 100).

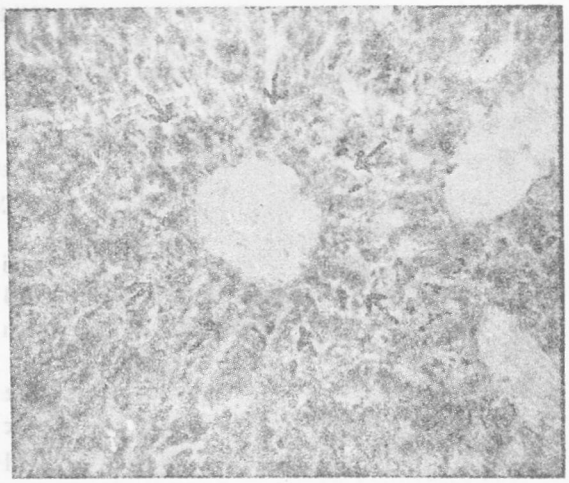

Fig. 3 : Proliferation of oval cells in the periportal zones (H & E) X 100.

whole liver after PH has been reportad to be 28 days. In order to study the histological changes in induced carcinogenesis even before complete regeneration of live itself the rats were killed on 15th day of carcinogen introduction. The histological preparations revealed presence of foci numbering 40 to 50/cm² of the section (Fig. 1). Visibility of necrotic hepatocytes in the area of the terminal hepatic venules was observed. The necrotic hepatocytes were gradully replaced by cellular proliferation. Though the normal acinen architecture was restored yet different hisotological changes such as proliferation of a new oval cells in the portal traids and in the peripheral zones were observed (Fig. 2). No evidence of ongoing necrosis was observed. Occasionally nuclear plemorphism was noticed in hepatocytes and basophilic clumping of the hepatocytes was of frequent occurrence (Fig. 2 and 3).

DISCUSSION

The appearance of foci in gross histological section (Fig. 1) induced by DEN has been attributed to the initial stage of carcinogenesis. According to Henry et. al. (1978) the foci are group of enzyme dificient cells particularly AT Pase. A similar number of foci as observed in our studies (40-50 foci cm²) has been reported during dose fractionation studies of DEN by Dennis et. al. (1976) at the same dose used by us.

The causation of necrotic hepatocytes could be explained due to toxic action of carcinogen (DEN) at dose level of 200 mg/kg body weight and surgical implications at the time of PH. Ongoing necrosis could not be found further because the liver has got greater capability of regeneration (Higgins and Anderson, 1931) and hence the hepatocytes proliferated and gradually replaced the necrotic hepatocytes. The restitution of the normal aciner architecture was another feature (Fig. 3) Eventually during proliferation of the cells the normal acinar architecture was also formed. The most significant change observed on 15th day (Fig. 3) is proliferation of few ovel cells represeting one set of hepatic carcinogenesis which according to Benito et. al. (1980) arose essentially due to irrevesible changes in very small number of hepatocytes due to somatic mutation and plays an important role in the sequential genesis of hepatocellular carcinoma. A similar observation has also been reported by Cairns (1975). Basophilic clumplng of hepatocytes cytoplasm observed after two weeks of PH was due to the reaction of carcinogen with cell constrtuents (Borenfreund et. al 1978, Sinha, 1983). Frederic et. al. (1979) has also observed production of basophilic cells after interaction of the carcinogen with the hepatocytes and the clumping of hepatocytes cytoplasm.

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