α-FETOPROTEIN SYNTHESIS IN RAT LIVER AFTER A SINGLE INJECTION OF THE HEPATOCARCINOGEN 4-DIMETHYLAMINOAZOBENZENE AND ITS NONCARCINOGENIC HOMOLOG 4-DIETHYLAMINOAZOBENZENE

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KEY WORDS: Carcinogens; 4-dimethylaminoazobenzene; noncarcinogenic homologs; liver; α-fetoprotein.

To characterize changes taking place during malignant transformation of cells the study of the action of carcinogens on target tissues long before the appearance of tumors in them is of the greatest importance. The difficulty of such investigations is the detection of true carcinogenic action, for most of the changes taking place in the early stages of carcinogenesis are not specific in character and are due to the toxic action of the carcinogens. One possible approach to the study of this problem is by studying early changes in a target tissue after a single injection of carcinogens [5], which can be regarded as the primary effect of the carcinogens. A single injection of the hepatocarcinogen 4-dimethylaminoazobenzene (DAB) into rats leads to changes in the synthesis of tissue-specific enzymes in the liver, a shift of the isozyme composition, antigenic simplification, and synthesis of heteroorganic antigens, in a similar manner to what takes place in hepatocellular tumors [4, 6]. However, unlike tumors induced by a single injection of a carcinogen, the changes are reversible. It is therefore interesting to study the synthesis of embryonic α-fetoprotein (AFP), which is known to be characteristic of hepatocellular tumors, in the liver of rats receiving a single injection of DAB and of its noncarcinogenic homolog 4-diethylaminoazobenzene (4-diethyl-AB).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-180 g. DAB and 4-diethyl-AB, dissolved in sunflower oil, were injected intraperitoneally in a single dose of 250 mg/kg body weight. The rats were killed by decapitation 1, 4, 7, 12, 18, 20, and 30 days after the injection. Pieces of liver were fixed and embedded in paraffin by Gleiberman's method [2]. AFP was detected by the indirect immunofluorescence method with rabbit serum against rat AFP (anti-AFP serum).* Sections of the liver of intact rat embryos and adult rats were used as the control. The specificity of the reaction was verified with serum of intact rabbits and also by exhaustion of the anti-AFP serum by a preparation of rat AFP polymerized with glutaraldehyde. Luminescent serum against rabbit globulin (prepared by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) was exhausted before the experiment with an acetone powder of liver.

When animals are fed on a diet containing carcinogens the earliest changes observed in the liver are degenerative changes among the hepatocytes with the appearance of foci of proliferation of oval cells [1, 9]. Oval cells appear 3-4 weeks after the beginning of feeding with carcinogens, and about at the same time AFP appears in the serum [12].

Degenerative changes in the hepatocytes were observed as early as 24 h after a single injection of DAB in the central zones of the hepatic lobules, whereas hepatocytes in the portal zones appeared unchanged. After

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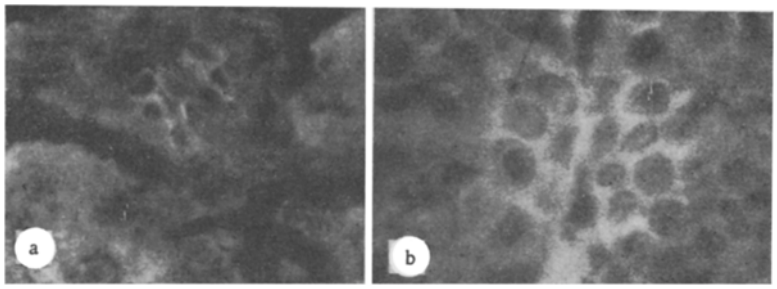

Fig. 1. Detection of AFP in rat liver after a single injection of DAB. Immunofluorescence test with anti-AFP serum. a) Liver on 7th day after injection of DAB; b) on 12th day after injection of DAB. 600×, water immersion.

4 days, foci of oval cells similar to those described in the rat liver during prolonged feeding with carcinogen appeared in the portal zones of the hepatic lobules. On the 7th day, the foci of oval cells attained their largest size – up to 360 μ in diameter. After 12 days, larger polygonal cells, with more cytoplasm than the oval cells, appeared at the periphery of the foci. In the literature such cells are described as basophilic hepatocyte-like cells [1] or transitional cells [10]. The morphological picture of the liver 20 days after injection of DAB was no longer distinguishable from that of the liver of intact animals.

Most cells in rat embryonic liver synthesized AFP. In the intact adult rat liver no AFP could be found. Four days after a single injection of DAB, when the appearance of foci of oval cells was noted for the first time, no AFP could be found. However, after the 7th day some cells in these foci began to synthesize AFP, as revealed by specific luminescence evoked by anti-AFP serum and localized in the cytoplasm of single oval cells or of their clusters (Fig. 1a). The strongest luminescence was observed 12 days after injection of DAB, in both oval and transitional cells (Fig. 1b). It must be emphasized that the luminescence detected in the rat liver sections must be regarded as specific, for preliminary exhaustion of the anti-AFP serum with the AFP preparation led to disappearance of the luminescence. On the 18th day the intensity of the luminescence was appreciably reduced, and after the 20th day it was virtually undetectable.

According to statements in the literature, after exposure of mice to carbon tetrachloride, AFP was synthesized by hepatocytes located at the periphery of the region of necrosis [3]. In the present experiments a single injection of DAB did not cause the formation of visible foci of necrosis in the rat liver, and this was probably why AFP synthesis was not detected in the hepatocytes. The findings indicate that a single injection of DAB causes the appearance of AFP only in foci of oval or transitional cells.

Prolonged feeding of rats with noncarcinogenic homologs of DAB (2-methyl-DAB and p-aminoazobenzene) does not lead to the appearance of AFP in the blood serum [8]. After a single injection of 4-diethyl-AB, a noncarcinogenic homolog of DAB, however, changes similar in their general features to those caused by the action of DAB were observed in the rat liver, namely: the appearance of foci of oval cells one day after injection (i.e., a little earlier than after injection of DAB); AFP synthesis in these foci of the 4th day; termination of the synthesis by the 2nd day, when the morphological picture of the liver of the rats receiving 4-diethyl-AB was indistinguishable from that of the liver of intact animals. AFP synthesis in the rat liver after administration of different carcinogens is known to depend on the dose of the agent given [11]. This may probably be connected with the fact that under the experimental conditions used, when the rats were given a single injection of relatively large doses of 4-diethyl-AB, it was possible to detect AFP synthesis.

The similarity between the changes induced by the carcinogen and its noncarcinogenic homolog, and also the dynamics of AFP synthesis, suggest that the oval and transitional cells are embryonic liver cells which arise in response to the harmful action of these two compounds and, after undergoing further differentiation, they replace the injured hepatocytes. The possibility cannot be ruled out that these morphological changes in the rat liver are due to a toxic action. Evidence in support of this view was obtained by Khodosova [6], who showed on the same model that similar changes in certain biochemical characteristics of the rat liver take place under the influence of a single injection of DAB and of 4-diethyl-AB. At the same time it should be noted that, when this same method was used, only DAB caused the characteristic changes of hepatocellular tumors such as antigenic simplification and divergence [4]. In the writers' view, all these observations serve to illustrate the well-known concept of the diversity of the pathways of disturbance of biosynthesis during carcinogenesis [7].

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EXPERIMENTAL CARCINOGENESIS OF THE BLADDER FOLLOWING ADMINISTRATION OF FREUND'S ADJUVANT AND LEVAMISOLE

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The object of this investigation was a histological and electron-microscopic study of the effect of Freund's complete adjuvant (FCA) and of levamisole on the early and late stages of experimental carcinogenesis in the urinary bladder.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar rats weighing 150-180 g. Throughout the experiment the animals were given a 0.05% aqueous solution of N-butyl-N-butanol(4)-nitrosamine, a known urotropic carcinogen, to drink. The animals were divided into six groups, with 20 rats in each group.

The animals of the first three groups were used to study the action of FCA and levamisole in the early stages of carcinogenesis, and those of the remaining three groups for the same purpose in the late stages.

The animals of group 1 (control) received the carcinogen only. In group 2, twice during the 10 days before the beginning of the experiment and twice during the 20th-30th days of carcinogenesis, the rats received a subcutaneous injection of 0.1 ml FCA in the plantar surface of the hind limbs. The rats of group 3 received an intraperitoneal injection of 3 mg/kg levamisole (Decaris, from Richter, Hungary), diluted in sterile physiological saline, daily for the 10 days before the beginning of the experiment and during the 20th-30th days of carcinogenesis. Five rats from each group were killed 5, 12, 20, and 40 weeks after the beginning of injection of the carcinogen. The animals of groups 4 and 5 received carcinogen only until they developed a carcinoma of the bladder after 32-34 weeks. At the 34th and 37th weeks of carcinogenesis the rats of group 4 were then

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