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ALTERATION OF ENZYME INDUCTION PATTERNS IN RATS TREATED WITH THE CARCINOGEN DIMETHYLNITROSAMINE

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Dimethylnitrosamine (DMNA) has been shown to be carcinogenic in various species of laboratory animals (1,2,3). Magee and Barnes (1) have shown that chronic treatment with DMNA in low doses produces a high incidence of hepatomas in rats. The mechanism of carcinogenesis seems to involve conversion of DMNA to an active alkylating agent (4). Labeled DMNA has been found to interact with DNA and RNA in tissues undergoing carcinogenic changes (5,6).

Alterations in the activity and inducibility of various enzyme systems have been reported to occur in hepatomas produced by various chemical carcinogens (7,8,9). Attempts have been made to utilize certain of these slow growing neoplasms designated as "minimal deviation" hepatomas (10) to explore and determine the early biochemical lesions which occur during the development of a neoplastic cell from a normal cell. The results reported in this paper represent data obtained while studying the alterations in various enzyme systems in livers from rats undergoing chronic treatment with low, carcinogenic doses of DMNA.

Methods

Male albino rats (Holtzman), weighing 50-60 grams, were started on the DMNA regimen. Dimethylnitrosamine (Eastman Kodak) was redistilled (BP = 150°) prior to use. DMNA was dissolved in the drinking water (contained in amber bottles) as an 0.002% solution and given continuously. The DMNA solution was

prepared twice a week, and the total water consumption was measured at various time intervals during the course of treatment. The average dose of DMNA per animal was 2 mg/kg/day. Animals were fed ad lib (Purina Laboratory Chow).

Hexobarbital sleeping times were performed by injecting 125 mg/kg of drug intraperitoneally and recording the time until the animals regained the righting reflex. Some of the animals were pretreated with phenobarbital (75 mg/kg) for 4 days prior to determination of hexobarbital sleeping times in order to determine inducibility of the drug-metabolizing enzyme system.

Induction of tryptophan pyrrolase (TP) with hydrocortisone was attempted in DMNA-treated rats at various times after initiation of DMNA treatment. DMNA solutions were replaced with untreated drinking water 12 hours prior to the experiments. Since an LD_{50} dose of DMNA (27-41 mg/kg) is completely metabolized in less than 24 hours by the rat in vivo (11), it was assumed that no unchanged drug was present at the time of the experiments. Both control and DMNA-treated rats were injected i.p. with hydrocortisone sodium succinate (40 mg/kg). Some of the rats were subsequently injected i.p. with 5 mg/kg of actinomycin D in propylene glycol (0.5% solution) or with propylene glycol alone. Animals were sacrificed by cervical dislocation, and the livers were immediately excised and frozen on dry ice. Tissue samples were stored at -60° for 24-48 hours prior to analysis. The livers were homogenized with a Waring blendor in 7 volumes of 0.14 M KCl containing 0.0025 N NaOH, and tryptophan pyrrolase activity was assayed in the whole homogenate by measuring the quantity of kynurenine produced according to the method of Feigelson and Greengard (12).

Results

The metabolism of hexobarbital in vivo by normal and DMNA-treated rats is shown in Table 1. Sleeping times for both non-induced and phenobarbital-pretreated animals are given. There was no increase in sleeping times during the duration of treatment with DMNA, indicating that the enzyme system for the metabolism of hexobarbital was still intact. However, induction of the drug-metabolizing enzyme system was diminished after 86 days of DMNA treatment.

TABLE 1

Inducibility of Hexobarbital Metabolism in Dimethylnitrosamine-treated Rats*

	Sleeping Time (minutes)				
	Control	Duration of DMNA Treatment (days)			
	-	13	34	86	114
No Pretreatment	76 ± 7 (n = 9)	90 ± 14 (n = 4)	67 ± 9 (n = 5)	37 ± 6 (n = 3)	52 ± 3 (n = 3)
Phenobarbital (75 mg/kg)	14 ± 2 (n = 6)	14 ± 5 (n = 3)	16 ± 1 (n = 4)	80 ± 46 (n = 3)	65 ± 36** (n = 2)

* Animals were injected i.p. with hexobarbital sodium (125 mg/kg), and the time to regain the righting reflex was recorded. Phenobarbital was injected i.p. for 4 days prior to assay of sleeping times. Values are expressed as mean ± standard error.

** Mean ± range.

The induction of tryptophan pyrrolase by hydrocortisone was also studied in DMNA-treated rats. Figure 1 depicts the kinetics for the metabolism of

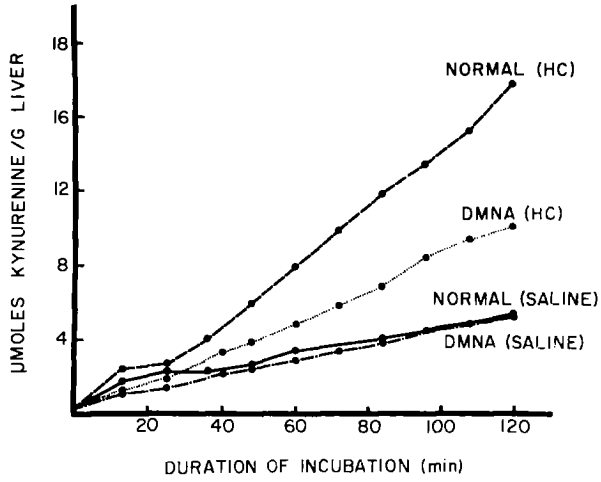

FIG. 1

Kinetics of the induction of tryptophan pyrrolase in normal and DMNA-treated rats. Animals were sacrificed 4 hours after injection of saline or hydrocortisone (HC) and TP activity was assayed in duplicate samples of liver homogenate at each time. DMNA rats were treated for 59 consecutive days prior to the experiment.

tryptophan in liver homogenates from normal and DMNA-treated rats 4 hours after injection of saline or hydrocortisone.

In both cases the level of enzyme activity was elevated after hydrocortisone, and the metabolism of tryptophan to kynurenine was linear for 120 minutes; but the level of induction of the enzyme was somewhat diminshed in the DMNA-treated rats.

In order to determine whether the induction of TP was achieved via an alteration of a "repressor" system as postulated by Garren et al. (13) and whether this "repressor" system was intact in the DMNA-treated animals, the inducibility of TP by hydrocortisone was assayed in normal and DMNA-treated animals before and after injection of actinomycin D. Figure 2 shows the induction pattern for the normal animals. Maximum enzyme activity occurred 4 hours

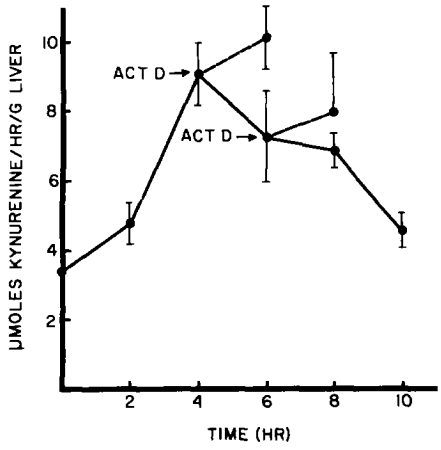

FIG. 2

Induction of tryptophan pyrrolase by hydrocortisone in normal rats. Kynurenine production was measured in duplicate samples of liver homogenate from 4-6 animals for each point. Actinomycin D (5 mg/kg) in propylene glycol or propylene glycol alone was injected i.p. 4 and 6 hours after administration of hydrocortisone. Administration of propylene glycol alone did not significantly alter TP activity in the hydrocortisone-treated rats. Results are expressed as mean ± S.E.

after injection of hydrocortisone. When actinomycin D in a dose (5 mg/kg) which abolishes all hepatic RNA synthesis with the exception of a small quantity of transfer RNA (14) was administered to hydrocortisone-treated rats at the peak of enzyme activity, a secondary stimulation of TP was observed. When actinomycin D was administered 6 hours after hydrocortisone, TP activity was not markedly different from that of animals treated with hydrocortisone alone.

In contrast to these observations, the induction of TP by hydrocortisone in DMNA-treated rats followed a more sluggish time course (Fig. 3). Peak enzyme activity occurred 6 hours after hydrocortisone. In addition, administration of actinomycin D did not produce a secondary stimulation of TP when injected 4 or 6 hours after hydrocortisone.

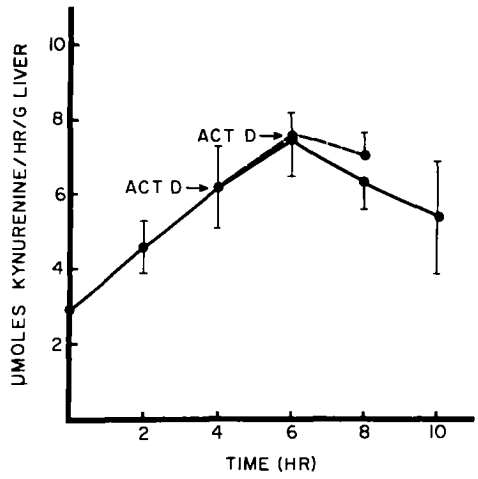

FIG. 3

Induction of tryptophan pyrrolase by hydrocortisone in DMNA-treated rats. Experiments were performed as described in the legend for Fig. 2. Rats were treated with DMNA for 78-93 days prior to the experiments.

It should be noted that the livers taken from rats treated with DMNA for 114 days showed "regenerative nodules characterized by large hyperchromatic cord cells with pleomorphic nuclei and an occasional mitotic figure" (a report from the Pathology Department of The University of Michigan). No malignant neoplasm was observed. These changes are similar to those described by Heath and Magee (15) as being typical of precancerous livers in DMNA-treated animals.

Discussion

Dimethylnitrosamine has been shown to produce a high incidence of liver neoplasms after prolonged oral administration (1,15). The liver tumors are mainly hepato-cellular carcinomas. All stages from liver cell adenomas to highly anaplastic hepato-cellular carcinomas have been noted. Some sarcomas are produced, and rarely carcinomas arising from intrahepatic bile ducts occur. Occasionally lung and kidney tumors have been observed, but these usually occur at higher dose ranges than those necessary to produce hepatomas.

Adamson and Fouts (7) have reported that the transformation of normal liver into hepatoma is accompanied by a loss of certain drug-metabolizing enzymes in the microsomal fraction. This change did not occur during the "precancerous" stage in livers of rats treated with 4-dimethylaminoazobenzene (DAB) but was observed only in the neoplastic tissue itself. The metabolism of hexobarbital in homogenates of livers taken from DAB-treated rats did not change significantly during 7 months of drug treatment (7). The data presented in this paper indicate that the *in vivo* metabolism of hexobarbital is not diminished in rats treated with DMNA for a period up to 114 days. However, the ability of the hexobarbital-metabolizing enzyme system to be induced by phenobarbital decreased after 86 days of DMNA treatment. This suggests that even though the hexobarbital-metabolizing system is intact in DMNA-treated rats, a portion of the control or "repressor" system responsible for regulating the level of the drug-metabolizing enzyme system is lost in precancerous liver.

The influence of substrate and adrenocortical hormone administration on the regulation of enzyme levels in rat hepatomas has recently been reviewed

(8,9). In general, induction of tryptophan pyrrolase did not occur in "minimal deviation" hepatomas after administration of cortisone acetate to tumor bearing hosts (8). Induction of the enzyme by tryptophan appears to be variable depending on the growth rate of the hepatoma (9). Garren et al. (13) observed a secondary stimulation of tryptophan pyrrolase in hydrocortisone-treated, adrenalectomized rats after administration of actinomycin D (5 mg/kg). These authors concluded that the regulation of TP is under the influence of a cytoplasmic repressor system which depends on continued RNA and protein synthesis and that the "repressor" has a more rapid rate of turnover than the mRNA needed for enzyme synthesis. We have observed in precancerous liver of DMNA-treated rats that the characteristics of TP induction are somewhat different from normal liver. The peak in enzyme activity is delayed by 2 hours in DMNA-treated liver, and no secondary stimulation was observed with actinomycin D, suggesting an alteration in the mechanisms regulating the level of tryptophan pyrrolase activity in precancerous liver.

The data reported here suggest that the repressor mechanisms involved in the control of enzymic activity are modified in tissue undergoing neoplastic change. This loss of the ability to regulate enzymes may reflect a generalized alteration in cellular control mechanisms during the transformation of a normal cell into a cancer cell. Investigation of alterations in other control mechanisms during carcinogenesis in DMNA-treated rats is being undertaken.

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