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Diethylnitrosamine-Induced Changes in Mouse Liver Morphology and Function[1] (39492)

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When rats and mice of various strains are exposed to the carcinogens, dimethylnitrosamine (DMN) or diethylnitrosamine (DEN), over prolonged time periods a number of proliferative and neoplastic changes occur in the liver. Parenchymal cell tumors, either hepatocellular carcinoma or hepatoma, have been reported (1-3) usually preceded or accompanied by hyperplastic nodule formation. In other investigations the predominant tumors have been cholangiocarcinomas or hemangiomas (4, 5). Bile duct proliferation to a greater or lesser extent occurs in all rats and mice treated with one of these dialkylnitrosamines (1-3).

Complex interactions occur between the dialkylnitrosamines and drugs which alter the activity of the liver drug-metabolizing enzyme systems (DMES). For example, phenobarbital administration which increases the activity of liver DMES enhances the mutagenic effects of the dialkylnitrosamines (6), but diminishes their neoplastic potential (7). Indeed, the acute administration of DMN or DEN to rats or to mice decreases liver DMES activity (8-10), a seemingly paradoxical effect for chemicals whose activation requires metabolism by these enzyme systems (11, 12). Whether a comparable depression in liver DMES activity accompanies long term administration of low doses of DMN and DEN is not known.

This report describes the effects on hepatic morphology of DEN administration over periods of 10 to 24 weeks to adult, male Swiss mice. Hexobarbital sleeping times and the response to a large dose of carbon tetrachloride have been used as *in vivo* indicators of the status of the liver DMES.

Methods. Adult male Swiss mice (COBS CD-1, obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 25 to 30 g were maintained 5 per cage and permitted constant access to food and water. DEN was added to the drinking water of several groups of mice to provide a concentration of 4 mg/100 ml. Phenobarbital sodium was incorporated in the drinking water of other groups of mice to provide a concentration of 30 mg/100 ml. After 10 days of exposure to phenobarbital, DEN in a concentration of 4 mg/100 ml was additionally added to the drinking water and 21 days later the phenobarbital sodium concentration was increased to 45 mg/100 ml. The reasons for using this dosing schedule for phenobarbital have been described in detail (13).

Hexobarbital sleeping times were determined in control and DEN-treated mice 10 and 24 weeks after the start of the experiments and in mice treated with phenobarbital plus DEN at 10 weeks after the start of the administration of DEN. Mice were killed 3 days after hexobarbital sleeping times had been determined, complete autopsies were performed, and segments of liver were fixed in 10% buffered formaldehyde solution. All sections were stained with hematoxylin eosin and, where appropriate, slides were stained with the Masson trichrome stain and with Wilder's reticulin stain. Frozen sections were stained with oil red O.

A preliminary observation suggested that DEN-treated mice were unusually resistant to the acute effects of CCl_4. The following experiments were performed to substantiate this observation. Fourteen control mice, seven mice treated with DEN for 10 weeks, and eight mice treated with DEN for 24 weeks were given CCl_4 in corn oil by gavage to provide a dose of CCl_4 of 0.5 μl per gram of body weight. Experience in this laboratory indicates that this dose is approximately

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the LD_{50} for CCl_4 in mice which have not received any other drugs or chemicals. Mice were observed for lethality and morbidity over a period of 42 hours and the surviving mice were then killed and autopsied, and segments of liver were taken for light microscopy. The malevolent signs associated with morbidity are difficult to describe in mice. However, overt manifestations such as "a sick appearance," lordosis, and sluggish motor responses to normal stimuli were taken as evidence of morbidity. Another criterion of CCl_4 effects was the presence of an obviously enlarged yellow or cream-colored liver.

Results. Gross changes in the livers of mice treated with DEN for 10 weeks were not remarkable; the livers of DEN-treated mice tended to be firmer than the control specimens. In mice given DEN for 24 weeks, the livers generally presented a granular surface, and nodules which were distinct and could be considered grossly to be tumors were observed on 10 of 27 livers from mice given DEN for 24 weeks. In five of these mice, nodules were solitary and clearly hemorrhagic. There were no tumors in other body tissues examined—lungs, GI tract, kidneys, urinary bladder, pancreas, and brain.

The histologic appearance of liver tissue from mice given DEN for 10 weeks was comparable to that described by Clapp and Craig (3). Some hepatocytes tended to be enlarged with large nuclei containing coarsely stained chromatin. The characteristic change seen in all sections was oval cell proliferation, presumably the result of biliary duct cell hyperplasia. By 12–14 weeks of treatment with DEN, bile duct proliferation and microcyst formation had become prominent and a few scattered necrotic hepatocytes also were present in most sections. Foci of inflammatory cells, mainly lymphocytes, were present in all sections.

The addition of phenobarbital did not basically modify the effects of 10 weeks of exposure to DEN on liver morphology. There appeared to be even more extensive oval cell proliferation in some sections of liver segments taken from mice treated with phenobarbital and DEN when compared with liver tissue from mice treated only with DEN for 10 weeks.

Bile duct proliferation and cyst formation had become widespread, even overwhelming, in livers from mice treated with DEN for 24 weeks (Fig. 1). The hepatic parenchyma often was divided into nodular masses (Fig. 1), but there was scant deposi-

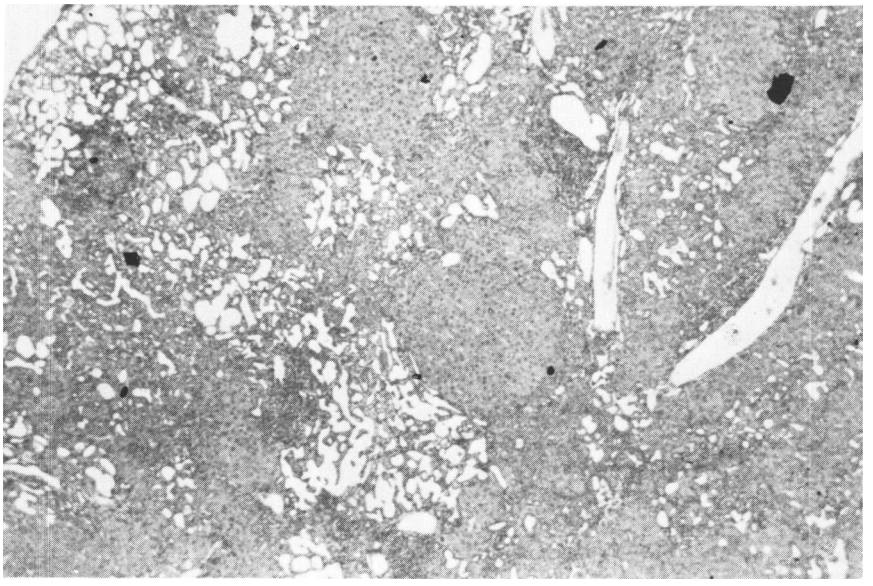

FIG. 1. Liver section from mouse given DEN in the drinking water for 24 weeks, showing extensive proliferation of bile ducts and nodule formation. ×25.

tion of collagen. The granular appearance of the liver, grossly giving an appearance of cirrhosis, resulted from the extensive proliferation of bile ducts and hepatic nodule formation rather than from deposition of fibrous tissue. A number of hepatocytes in each section contained bizarre, enlarged nuclei with prominent nuclear inclusions (Fig. 2). Scattered throughout the sections were foci of inflammatory cells, primarily lymphocytes (Fig. 2).

Many of the tissue nodules were composed of hyperplastic cells and in two sections the cytology of the cells within the nodules resembled hepatomas. No hepatocelluar carcinomas were found. The only well-defined neoplastic changes seen were one hemangioma, and in two sections, tissue suggestive of cholangiocarcinoma. The hemorrhagic surface nodules were blood-containing cysts surrounded by proliferating bile ducts.

Hexobarbital sleeping times were significantly increased in mice treated with DEN for 10 weeks (Table I), an effect which was markedly enhanced in mice treated with DEN for 24 weeks (Table I). Hexobarbital sleeping times in mice treated with both DEN and phenobarbital were similar to controls (Table I).

Carbon tetrachloride administration resulted in the death of 6 of 14 control mice within 26 hr after CCl_4 had been given (Table II). Morbidity was observed in each of the remaining eight mice and fatty liver was present in all 14 mice (Table II). Histologically, liver sections from CCl_4-treated control mice showed the characteristic effects of CCl_4—extensive central lobular necrosis and widespread fatty change. There were no deaths, morbidity, nor fatty livers in any of the DEN-treated mice given carbon tetrachloride (Table II). None of the characteristic histologic changes of CCl_4 was observed in most sections from DEN-treated mice. The only histologic evidence of some effects of CCl_4 was hepatocytes showing a ballooning-type degeneration, which were scattered throughout the sections of livers taken from four of the mice treated with DEN for 24 weeks.

Discussion. Long-term exposure of male Swiss mice to DEN produced an adaptative response characterized by biliary ductule cell hyperplasia which in later stages resulted in widespread bile duct proliferation. Hepatic parenchymal cells appeared to be much less affected by the nitrosamine, for well-defined evidence of hepatocytic response was not seen until at least 12 weeks

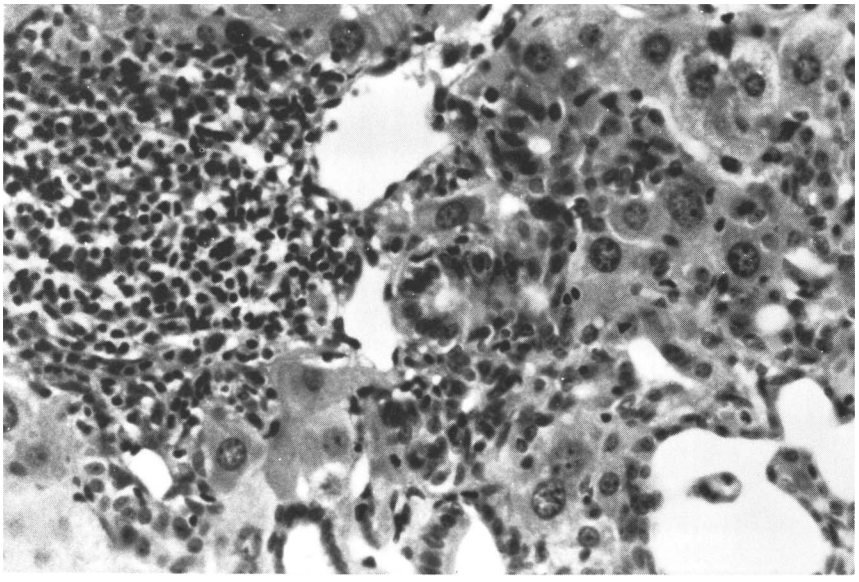

FIG. 2. Higher magnification showing details of bile duct proliferation and an aggregate of lymphocytes. × 100.

TABLE I. HEXOBARBITAL SLEEPING TIMES IN CONTROL MICE AND IN MICE TREATED WITH DIETHYLNITROSAMINE (DEN) AND WITH PHENOBARBITAL AND DIETHYLNITROSAMINE (PB-DEN)[a]

Group	N[b]	Sleeping times (min)
Control	9	32.4 ± 3.6
DEN (10 weeks)	13	61.2 ± 4.7[c]
PB-DEN (10 weeks)	8	33.0 ± 3.5
Control	8	30.6 ± 2.1
DEN (24 weeks)	13	160.6 ± 17.4[c]

[a] The results are expressed as the mean ± one standard error of the mean. Hexobarbital sodium was dissolved in 0.154 *M* NaCl solution and administered intraperitoneally at a dose of 100 μg/g of body weight.
[b] Number of mice in each group.
[c] $P < 0.001$.

TABLE II. EFFECT OF AN ACUTE DOSE OF CCl_4 IN CONTROL MICE AND IN MICE TREATED WITH DIETHYLNITROSAMINE (DEN)

Group	Deaths	Morbidity within 42 hr[a]	Fatty liver at 42 hr[a]
Control	6/14	8/8[b]	8/8[b]
DEN (10 weeks)	0/7	0/7	0/7
DEN (24 weeks)	0/8	0/8	0/8

[a] Forty-two hours after the administration of CCl_4.
[b] Surviving mice.

of exposure to DEN. The effects of DEN on hepatic morphology in this strain of Swiss mice were similar to the responses to DMN by mice of the RF strain (5), although the incidence of neoplastic transformation was very low in the Swiss mice. Foci of lymphocytic infiltration were a prominent feature of the adaptative responses of the liver to chronic exposure to DEN. Possibly, this indicates a high level of immunosurveillance which may be related to the low incidence of neoplasia.

CCl_4 must be metabolized by liver DMES to more highly reactive derivatives, which are the ultimate hepatotoxins (14–16). The striking resistance by DEN-treated mice to the hepatotoxic effects of CCl_4, therefore, probably reflected a marked diminution in liver DMES activity. If liver DMES activity were markedly reduced by exposure to DEN it would be logical to assume that DEN-treated mice would show minimal effects to doses of CCl_4 which produced severe hepatotoxicity in untreated mice. The marked increases in hexobarbital sleeping times support the conclusion that DEN progressively decreased liver DMES activity. That there was continued morphologic response to DEN despite the apparent reduction in liver DMES activity suggests either that a minimal level of liver DMES activity was present, sufficient to activate DEN, or that systems other than those associated with the liver microsomes activate the dialkylnitrosamines (17). The metabolism of exogenous chemicals could also have been altered by the intense, widespread proliferation of bile ducts, but there is no information regarding such influences on drug metabolism and disposition.

The apparent cancellation by phenobarbital of DEN-induced increases in hexobarbital sleeping times could have been the result of simple addition of the stimulation by phenobarbital of liver DMES activity and its depression by DEN. However, phenobarbital treatment generally increased the acute toxicity of potential liver toxins and enhanced some of the effects of long-term administration of hepatotoxins (13). The interaction between phenobarbital and hepatotoxins on liver DMES activity when each is given concurrently over long time periods is unknown. Moreover, adaptative changes in liver ultrastructure and DMES activity occurred during long-term administration of phenobarbital to rats (18), while in some strains of mice, liver cell changes suggestive of neoplastic transformation accompanied chronic phenobarbital administration (19). Although phenobarbital prevented DEN-induced increases in hexobarbital sleeping times, corresponding reduction of DEN-induced morphologic changes at the light microscopic level did not occur.

Summary. Long-term administration of DEN to adult Swiss mice for periods of up to 24 weeks resulted in intense bile duct proliferation, formation of hepatic nodules, some of which appeared to be hyperplastic, and foci of lymphocytic infiltration. No hepatocellular carcinomas or well-defined hepatomas were found in these DEN-treated mice. The only tumors observed were one hemangioma, and in 2 sections, tissue suggestive of cholangiocarcinoma. The sig-

nificant increases in hexobarbital sleeping times and the striking resistance to the hepatotoxic effects of CCl_4 in DEN-treated mice indicated that long-term exposure to DEN resulted in a progressive inhibition of liver DMES activity. Phenobarbital administration concurrent with DEN prevented the decreases in hexobarbital sleeping times but did not alter the morphologic changes in the liver produced by DEN, at least at the light microscopic level.

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