The Sequential Analysis of Liver Cell Necrosis

Inhibition of Diethylnitrosamine- and Dimethylnitrosamine-Induced Acute Liver Cell Death by Posttreatment With Diethyldithiocarbamate

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Posttreatment with diethyldithiocarbamate (DEDTC) largely prevented the development of acute hepatocellular necrosis induced by diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) in male Fischer rats as monitored by the release of glutamate-pyruvate transaminase and sorbitol dehydrogenase into the serum and by histologic examination. Liver cell necrosis was evident with a dose of 25 mg of DEN/kg and was progressive with increasing doses of DEN. DEDTC (50 mg/kg; three times at 4-hour intervals) was given at 4 or 8 hours after the administration of DEN (100 mg/kg), time points at which at least 50% and 75%, respectively, of the administered DEN had disappeared from both the serum and liver. Under these conditions, DEDTC prevented liver cell necrosis, except for a few isolated single cells. Similar inhibition was also observed when DEDTC was given 4 hours after the administration of a necrogenic dose of DMN (20 mg/kg). DEDTC, when administered 4 hours after DEN, delayed the rate of clearance of DEN and of ethylation of DNA and RNA but did not significantly affect the total extent of ethylation of rat liver nucleic acids. These results offer further support for the multistep hypothesis for the development of liver cell necrosis. (Am J Pathol 1980, 99:159-174)

CELL DEATH is commonly observed as part of the toxic effects of many chemicals, both those in the environment and those used therapeutically. The liver is especially vulnerable in this respect, probably due in part to its versatility in metabolizing exogenous chemicals.

There is a growing suspicion that cell death in some organs, including the liver, may be a multistep process with some steps specifically related to the nature of the individual etiologic agent and others common to many.[1-5] If this hypothesis is valid, it should be possible to influence the necrogenic process by treatment with appropriate modulators *after* the sequence has been initiated, ie, by "posttreatment."

During a study of the initiation of liver carcinogenesis by single doses of selected nitrosamines, such as diethylnitrosamine (DEN) and dimethylnitrosamine (DMN), and the possible importance of cell death in this proc-

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ess, it was observed that treatment of rats with diethyldithiocarbamate (DEDTC) some hours after the administration of the carcinogen can effectively inhibit the development of cell death and subsequent necrosis of hepatocytes. Under such circumstances, a considerable amount of the administered nitrosamine undergoes metabolism and interaction with cellular constituents before the possible protective agent is given. Previous work had shown that DEDTC or its parent compound disulfiram could prevent or delay liver cell necrosis if given *before* DMN,[6-8] DEN,[9] or CCl_4.[10,11]

The experimental observations relating to the histologic and biochemical effects of posttreatment with DEDTC after DMN or DEN and a beginning analysis of possible biochemical mechanisms are the subject of this article.

Materials and Methods

Animals

Male Fischer-344 rats (Microbiol Associates, Walkersville,Md) weighing 175–200 g were maintained on a 24% protein semisynthetic diet [12] (Bio-Serv Inc., Frenchtown, NJ) and a daily cycle of alternating 12-hour periods of light and darkness. The animals were given food and water *ad libitum* and were acclimatized to their environment for 1 week before the start of the experiment.

Chemicals

The purity of DEN (Eastman-Kodak Co., Rochester, NY) and DMN (Merck-Schachardt, Munich, Germany) were routinely checked prior to use by ultraviolet spectrophotometry and gas chromatography. The sodium salt of diethyldithiocarbamate (DEDTC) and all other biochemicals were obtained from Sigma Chemical Co., St. Louis, Missouri. ^{14}C-Diethylnitrosamine (sp act 100 mCi/mmole) was purchased from NEN, Montreal, Quebec.

Light Microscopy

The animals were killed under mild ether anesthesia. Sections of liver were fixed in Carnoy's fixative (ethanol/chloroform/acetic acid 6:3:1). After 3–4 hours the tissues were transferred to 95% ethanol for 24 hours, then 70% ethanol, processed and stained with hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS).

Determination of Serum Enzymes

Blood was withdrawn from the abdominal aorta. Serum glutamate-pyruvate transaminase (GPT) and sorbitol dehydrogenase (SDH) were assayed in duplicate according to methods described by Bergmeyer and Brent[13] and Gerlach and Hiby,[14] respectively.

Estimation of DEN in Blood and Liver

Blood was obtained from the abdominal aorta, and the excised liver was homogenized in 10% cold 0.9% NaCl solution. The samples were then extracted twice with three volumes of methylene chloride. Nitrosamine concentrations in the extracts were determined by gas chromatography (GLC) (F&M Scientific Model 402 Gas Chromatograph) using a column

packed with 10% FFAP supported on 80/10 Chromosorb W/AW (Supelco, Inc., Bellefonte, Pa). The GLC system was first standardized by the use of mixtures of known proportions of purified DMN and DEN. The lowest level of detection of the nitrosamine was 5–10 ng/ml of the sample.

Alkylation of Rat Liver DNA and RNA

A 10% homogenate of liver from rats after appropriate treatment following the administration of ^{14}C-DEN was prepared in 0.25 M sucrose containing 0.05 M tris, pH 7.4, 0.025 M KCl and 0.005 M $MgCl_2 \cdot 6H_2O$ (TKM) solution and was fractionated into nuclear and microsomal components according to the methods previously described.[15] Nuclear DNA was prepared by the method of Marmur.[16] Microsomal RNA was isolated by the modified procedure of Kirby and Cook.[17]

For measurement of radioactivity the purified DNA was hydrolyzed in 0.1 N HCl at 37 C for 18 hours or at 70 C for 1 hour, and RNA was hydrolyzed in 1 N HCl at 100 C for 1 hour. Radioactivity in hydrolysates was determined using an Intertechnique liquid scintillation spectrometer.

Analysis of ^{14}C-DEN-labeled liver DNA and RNA for 7 ethylguanine (7 EtG) was done by use of a Bondapak C-18 column in a Waters Model 6000A-UAK high-pressure liquid chromatographer eluting with 0.05 M ammonium formate buffer at pH 5.0. The radioactivity eluted with a standard 7 EtG was quantitated. Synthetic 7-EtG was prepared [18] and characterized with ultraviolet spectroscopy and paper chromatography.[19]

Results

Dose-Response Studies

The initial experiments were designed to determine the doses of DEN and DMN that were necrogenic to rat liver. Various doses ranging from 15 to 150 mg/kg of DEN and 5 to 30 mg/kg of DMN were administered intraperitoneally, and the rats were killed 3 days later. The extent of necrosis was assayed both by histologic examination and by measurement of serum SDH and GPT levels.

As shown in Table 1, the degree of induction of liver necrosis and of elevation of serum SDH and GPT are dose-dependent with both DEN and DMN. With the lowest dose of DEN (15 mg/kg) and two lowest of DMN (10 and 5 mg/kg), no liver cell damage was detectable by light microscopy and no elevation of either enzyme activity was seen in serum. With DEN, spotty necrosis in Zone 3 was seen with a dose of 25 mg/kg without a significant rise in enzyme levels. With DMN, necrosis and elevated enzyme activities were seen at a dose of 15 mg/kg. With larger doses of each nitrosamine, necrosis (Figures 1 and 3) and levels of serum enzymes increased in parallel.

The necrosis, when present, was always maximal in Zone 3 (Figures 1 and 3) and expanded into Zone 2 with increasing damage. As is well known,[20] DMN is more toxic and necrogenic than DEN on a weight and molar basis. With each agent, but especially with DMN, congestion with

Table 1—Induction of Acute Liver Cell Necrosis in Rats by Various Doses of Diethyl and Dimethyl Nitrosamines

Dose of nitrosamines administered (mg/kg)*	Extent of hepatic necrosis		
	Histologic evaluation†	Serum enzyme level (IU/1)	
		SDH	GPT
0.9% NaCl	0	4.6 ± 0.6 (15)‡	24.6 ± 1.9 (15)‡
DEN			
15	0	4.3 ± 0.4 (10)	30.1 ± 1.7 (10)
25	½+	4.8 ± 0.6 (6)	22.9 ± 3.3 (6)
50	1+	24.9 ± 3.0 (6)	40.0 ± 3.8 (6)
75	2+	47.0 ± 4.6 (10)	116.2 ± 10.1 (10)
100	3+	66.1 ± 7.0 (10)	270.0 ± 26.0 (10)
150	4+	165.3 ± 18.7 (6)	924.0 ± 138.0 (6)
DMN			
5	0	5.3 ± 0.8 (6)	ND
10	0	6.4 ± 0.9 (6)	ND
15	1+	18.2 ± 2.8 (6)	ND
20	3+	31.8 ± 4.6 (10)	275.2 ± 29.1 (6)
30	4+	98.4 ± 14.3 (10)	ND

* Animals were killed 3 days after the administration of the nitrosamines or 0.9% NaCl.
† Extent of necrosis was scored by histologic examination, taking 4+ to represent necrosis of Zones 2 and 3.
‡ Values are mean ± SE, and numbers in parentheses represent the number of rats used.
ND = not determined.

some hemorrhage was commonly seen in areas of most intense necrosis (Zone 3).

Inhibition of Nitrosamine-Induced Liver Necrosis by DEDTC

Having determined the necrogenic doses of DEN and DMN, the next experiment was conducted to examine whether DEDTC would influence the development of necrosis when given *after* the administration of each nitrosamine. Since multiple injections of DEDTC were necessary for optimal prevention of DMN-induced liver cell necrosis using pretreatment,[6] DEDTC was given three times at 4-hour intervals commencing at 4, 8, or 24 hours after the time of administration of nitrosamines. The rats were killed at 3 days.

The results presented in Table 2 show an almost complete protection against DEN or DMN-induced liver cell necrosis when the first dose of DEDTC was given 4 hours after the administration of either nitrosamine. Histologic examination showed no zonal necrosis, slight congestion, depletion of glycogen, and at most a few isolated dead cells in Zone 3 (Figures 2 and 4). Under these conditions, the levels of serum SDH and GPT were somewhat elevated above the control values (Table 2). Considerable pro-

Table 2—Effect of Posttreatment With Diethyldithiocarbamate (DEDTC) on DEN- and DMN-Induced Hepatic Cell Necrosis and Elevation of Serum Enzyme Levels

Experimental	Chemical	Administered dose (mg/kg)	DEDTC (mg/kg)*	Time of Post-treatment (hours)	Extent of hepatic necrosis: Histologic evaluation	Extent of hepatic necrosis: Serum enzyme level (IU/l) SDH	Extent of hepatic necrosis: Serum enzyme level (IU/l) GPT
1	0.9% NaCl	—	50 × 3	4	0	4.0 ± 0.5 (6)†	28.4 ± 2.5 (6)†
2	DEN	75	0.9% NaCl	4	2+	47.0 ± 4.6 (10)	116.2 ± 10.1 (10)
		75	50 × 3	4	0	22.3 ± 5.7 (6)‡	68.7 ± 7.4 (6)‡
		75	50 × 3	8	½+	25.6 ± 4.2 (6)	82.8 ± 6.5 (6)
3	DEN	100	0.9% NaCl	4	3+	66.1 ± 7.2 (10)	270.6 ± 26.0 (10)
		100	50 × 3	4	0	26.2 ± 3.4 (10)‡	107.1 ± 16.8 (10)‡
		100	50 × 3	8	½+	22.8 ± 2.8 (6)	147.7 ± 30.2 (6)
		100	50 × 3	24	2+	46.1 ± 3.9 (6)	166.5 ± 14.0 (6)
4	DEN	150	0.9% NaCl	4	4+	165.3 ± 18.8 (6)	924.5 ± 138.0 (6)
		150	50 × 3	4	1+	31.9 ± 4.1 (6)‡	196.1 ± 13.5 (6)‡
		150	50 × 3	8	2+	45.3 ± 4.8 (6)	301.5 ± 23.3 (6)
5	DMN	20	0.9% NaCl	4	3+	31.8 ± 4.6 (10)	275.2 ± 29.1 (6)
		20	50 × 3	4	½+	10.5 ± 1.0 (6)‡	91.3 ± 14.3 (6)‡

* DEDTC (50 mg/kg, 3 times at 4-hour intervals) or 0.9% NaCl was administered intraperitoneally at 4, 8, or 24 hours after the administration of the nitrosamines, and the rats were killed 3 days later.

† Values are mean ± SE, and the number in parentheses represents the number of rats used.

‡ Significantly different from groups that received nitrosamine + 0.9% NaCl ($P < 0.001$).

Table 3—Influence of DEDTC on the Incorporation of Radioactivity and the Formation of 7-Ethylguanine in Rat Liver DNA and RNA by ^{14}C-DEN

Treatment*	Time of death	DNA		RNA	
		Total radioactivity (pmole/mg nucleic acid)	7-ethylguanine formed (pmole/mg nucleic acid)	Total radioactivity (pmole/mg nucleic acid)	7-ethylguanine formed (pmole/mg nucleic acid)
^{14}C-DEN	4 hr	140.0	10.8	457.0	13.3
		64.2	8.5	325.2	19.0
		72.2	11.2	294.2	15.5
	8 hr	138.6	15.9	559.6	20.6
		149.2	23.0	815.0	29.7
	24 hr	261.0	29.4	2516.6	37.1
		339.5	47.5	4248.4	55.9
	28 hr	326.7	28.2	1440.8	34.8
		307.8	30.7	3206.2	32.4
^{14}C-DEN + DEDTC	28 hr	181.4	19.6	873.1	29.3
	48 hr	267.0	25.0	1594.8	ND
		252.5	29.3	2095.4	31.9
		222.4	17.4	1403.4	24.3

* The rats were given ^{14}C-DEN (50 μCi/100 mg/kg) and were killed at 4, 8, 24, or 28 hours later. In a second group, DEDTC (50 mg/kg, 3 times at 4-hour intervals) was administered 4 hours after the administration of ^{14}C-DEN, and the rats were killed 24 hours and 44 hours after the first injection of DEDTC. Other details are described in Materials and Methods. Each number is the value for one rat liver.

ND = not determined.

tection against liver cell necrosis was found also when DEDTC was given as long as 8 hours after the time of DEN injection. However, when DEDTC was administered at 24 hours (Tables 2 and 3), the protection against DEN-induced liver cell damage was minimal.

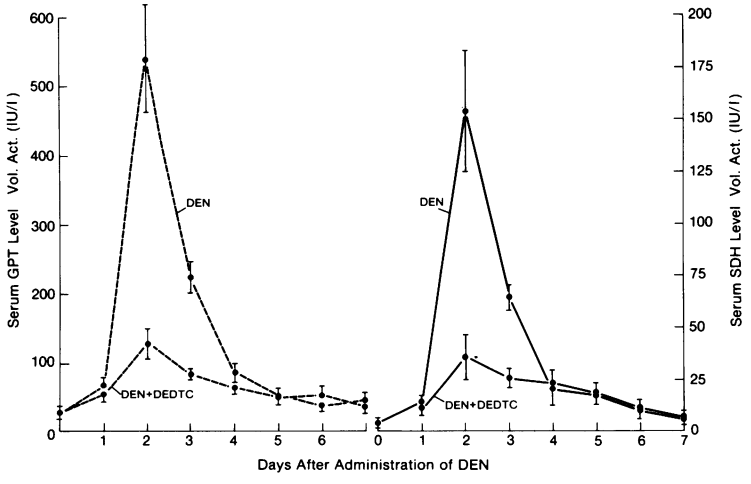
Although the above results indicate that posttreatment with DEDTC can effectively prevent liver necrosis induced by DEN when monitored at 3 days, they did not show whether the effect is merely one of delaying the process or truly preventing it. With this in mind, a time course study was carried out. DEDTC was administered at 4 hours after the injection of DEN (100 mg/kg), and the rats were killed from 1 to 7 days later. The sequential histologic analysis of the liver showed that with DEN treatment alone in the first 24 hours, minimal changes were observed, with loss of basophilia and glycogen depletion in the hepatocytes, especially in Zone 3. Maximal necrosis was seen on Day 3 with infiltration of inflammatory cells (Figure 1). Mitoses were seen in Zone 1, and complete replacement of Zone 3 necrotic cells occurred by about Day 7. As shown in Text-figure 1, the release of serum GPT and SDH in DEN-treated rats was greatest on Day 2 and returned to control values by Day 6. However, when DEDTC was given, a significant reduction in the release of both enzymes was seen

throughout the whole period. A similar inhibition in the development of necrosis was confirmed by histologic examination. The minor eosinophilia and glycogen depletion observed in hepatocytes on Day 3 did not progress into zonal cell death, and the liver was completely recovered by Day 5. A few animals given DEN plus DEDTC at 4 hours were followed for 14 days and showed no evidence of a more prolonged delay in the onset of necrosis after 7 days.

Effect of DEDTC on Clearance of DEN

Since pretreatment with DEDTC is known to inhibit the plasma clearance DMN, secondary to an inhibition of hepatic metabolism,[6] it became important to observe any effects of DEDTC on the clearance of DEN. DEN in general is somewhat more slowly metabolized than is DMN and could possibly be influenced more by posttreatment.

The results presented in Text-figure 2 show the disappearance curves of DEN from the blood and the liver in control rats and in rats given DEDTC 4 hours after 100 mg/kg DEN. By 4 and 8 hours after a single injection of DEN, about 50% and 75%, respectively, of the administered dose have disappeared from both blood and liver, when compared with the values at 5 minutes. The levels in blood and liver were undetectable at 24 hours. The clearance from both blood and liver was delayed by the administration of DEDTC at 4 hours. The 75% value was seen at 24 hours

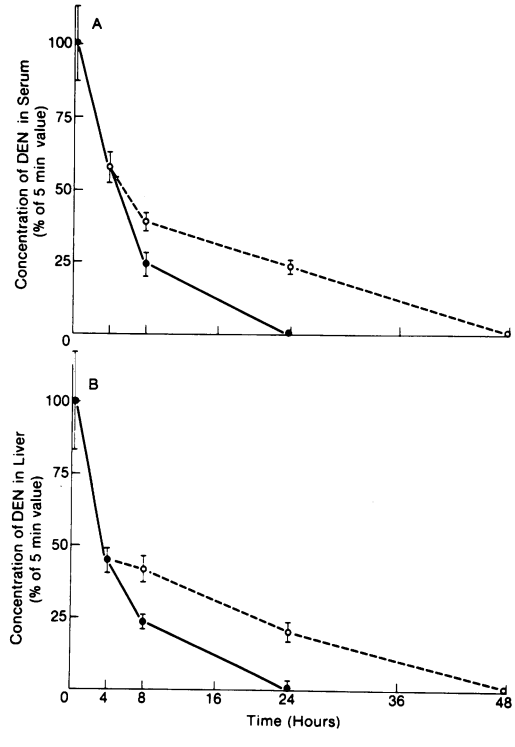


TEXT-FIGURE 1—Time course study on the effect of DEDTC on the release of enzymes GPT and SDH in serum induced by DEN (100 mg/kg). DEDTC (50 mg/kg, 3 times at 4-hour intervals) was begun 4 hours after DEN administration. Enzyme activities are expressed as volume activity (IU/1).

instead of 8 hours. No measurable levels of DEN in blood or liver were present by 48 hours.

Effect of DEDTC on Ethylation of Liver DNA and RNA

Since alkylation of cellular macromolecules is considered to be of major importance in the genesis of cell injury and of cancer by DMN and DEN,[20-24] it became of interest to determine the influence of post-treatment with DEDTC on the levels of labeling and of ethylation of some key cellular constitutents such as DNA and RNA. As shown in Table 3, the total labeling of DNA and RNA from the labeled ethyl groups of DEN and the amount of one of the major specific reaction products, 7 EtG, increase progressively with time up to 24 hours. The values then plateau for at least 4 hours. The administration of DEDTC delayed the total labeling and the formation of 7-EtG in both DNA and RNA. However,



TEXT-FIGURE 2—Influence of DEDTC on the disappearance of DEN from the circulating blood (A) and the liver (B). Groups of 3 rats were used. DEDTC (50 mg/kg, 3 times at 4-hour intervals) was administered 4 hours after DEN (100 mg/kg) treatment. The solid lines represent the levels of DEN in the serum and the liver of the controls, and the dotted lines represent the values in the rats post-treated with DEDTC. The values are the percentage of the 5-minute level. The 5-minute values in serum and in liver were 137.5 ± 10.8 μg/ml and 27.7 ± 4.7 μg/g, respectively.

the levels reached at 48 hours, by which time no DEN could be detected in either the liver or the blood (Text-figure 2), were within 20–25% of those at 24 hours in the controls. Thus, it appears that the interaction of the DEN with the liver DNA and RNA by 48 hours has reached a level not far removed from that seen in the DEN-treated controls, but it has taken longer to do so.

Discussion

It is evident from the results of this study that the administration of DEDTC several hours *after* the injection of DMN or DEN can protect to a major degree against the induction of acute hepatocellular necrosis. The protection is not merely due to delay but appears to be a valid prevention of cell damage.

DEDTC, a known free radical scavenger, inhibits the metabolism of several potentially toxic chemicals, including DMN[6,25] and CCl_4.[10,26] The protection against the toxic effects of these chemicals by pretreatment with DEDTC could be due to an inhibition or delay of their metabolic conversion to reactive toxic derivatives. Thus, pretreatment could lead to a protective effect at a very early step in the metabolic sequence leading to cell injury.

This explanation cannot be invoked in the case of DMN and post-treatment with DEDTC. At a dose of 20 mg/kg, DMN is completely metabolized within 4–5 hours to one or more methylating species and to formaldehyde, and the molecular targets for methylation, such as DNA, RNA, and protein, have reached plateau values for alkylation of esterification of their components.[20,22,27–29] It is generally considered likely that methylation of cellular components and/or formaldehyde are involved somehow in the subsequent steps leading to irreversible cell damage.[20,22] Thus, whatever DEDTC is doing in protecting against liver cell death, its effect must be exerted on some step or steps in the molecular pathogenesis of hepatic necrosis that follows the initial metabolic activation of DMN. The specific nature of such a postulated step or steps is unknown. Conceivably, it could be an altered molecule or macromolecule that is participating in a subsequent reaction that can be modulated by a scavenger such as DEDTC.

Any explanation of the findings with DEN of necessity must be more controversial. DEDTC is most effective in preventing DEN-induced liver cell damage when given at a time at which some DEN is still being metabolized and only minimally effective when given at 24 hours, when all the DEN has been cleared from the blood and liver and the ethylation of DNA and RNA has reached a plateau. One could argue, therefore, that

DEDTC is effective with DEN by inhibiting the metabolism of the remaining 50% or 25% at 4 or 8 hours, respectively. However, the inhibition is not persistent. The remaining DEN becomes metabolized later, as indicated by its subsequent clearance from the blood and liver and the increasing ethylation of liver DNA and RNA up to 48 hours. If some immediate metabolic product or an ethylated form of a cellular macromolecule were the immediate target for cell death, only a small fraction at most could be involved, since the final degree of ethylation quantitatively is not very different from that in the DEN controls in which obvious liver cell necrosis occurs.

Alternatively, the *rate of alteration* of a cellular product by ethylation or by acetaldehyde could be more important than the *extent of alteration* in the genesis of liver injury. Since repair of DNA "damage" does occur, it is possible that the slower rate of ethylation or other change could allow for a more extensive repair and thus be associated with less tissue damage. If this possibility is valid, and if DNA is a possible target macromolecule involved in cell death, the relevant repair must involve only a small proportion of the DNA, since the levels of ethylation in the DEDTC-treated animals at 48 hours are not very different from those in the plateau range in the controls.

Another possibility is on effect of DEDTC via a chelating action on Ca^{++}. There is increasing evidence that excess cellular Ca^{++} appears to be one common mediator of cell death [1,2,5,30–37] and that several protective agents, such as some antihistamines, ethylene diamine tetraacetate and phenergan, may exert their effect on cell death through an action on Ca^{++}.[1,2,5,33,35,36]

The results of the present study add further evidence toward the conclusion that the blood clearance of some chemicals, including nitrosamines, might be dependent upon their metabolism. Even though these are water-soluble small-molecular-weight compounds, DMN and DEN seem not to be excreted in a major way until metabolized in some manner. Also, these nitrosamines, when their clearance is inhibited or delayed, remain available for the continuation of their metabolism such that the levels of interaction with some cellular macromolecules are not very different from those under the more usual circumstances of uninterrupted metabolism. The mechanism underlying this interesting phenomenon remains to be clarified.

The findings in this study also add additional evidence in support of the hypothesis that the development of cell death in the liver with toxic chemicals may be a multistep process in which one or more steps beyond the initial activation to reactive derivatives can be modulated by the ad-

ministration of appropriate drugs or chemicals.[1,2,4,5,37] This thesis has an obvious practical implication in suggesting that it may be possible to mitigate the toxic effects of environmental chemicals in their continuing presence by effects on steps beyond the initial metabolic activation when these become better understood.

References

1. Judah JD, Ahmed K, McLean AEM: Possible role of ion shifts in liver injury. Ciba Foundation Symposium on Cellular Injury. Edited by AVS de Reuck, J Knight. Boston, Little, Brown and Co, 1964, pp 53–60
2. McLean AEM, McLean E, Judah JD: Cellular Necrosis in the liver induced and modified by drugs. Int Rev Exp Pathol 1965, 4:127–157
3. Magee PN: Toxic liver necrosis. Lab Invest 1966, 15:111–131
4. Farber E: Biochemical Pathology. Ann Rev Pharmacol 1971, 11:71–96
5. Farber JL: Reaction of the liver to injury: Necrosis. Toxic Liver Injury. Edited by E Farber, MM Fisher. New York, Marcel Dekker, 1979, pp 215–248
6. Abanobi SE, Popp JA, Chang SK, Harrington GW, Lotlikar PD, Hadjiolov D, Levitt M, Rajalakshmi S, Sarma DSR: Inhibition of dimethylnitrosamine-induced strand breaks in liver DNA and liver cell necrosis by diethyldithiocarbamate. J Natl Cancer Inst 1977, 59:263–271
7. Schmähl D, Krüger FW: Influence of disulfiram (tetraethylthiuramdisulfide) on the biological actions of N-nitrosamines. Topics in chemical carcinogenesis. Edited by W Nakahara, S Takayama, T Sugimura, S Ödashima. Tokyo, University of Tokyo Press, 1972, pp 199–235
8. Schmähl D, Krüger FW, Ivankovic S, Preissler P: Verminderung der Toxizität von Dimethylnitrosamin bei Ratten und Mäusen nach Behandlung mit Disulfiram. Arzneim Forsch 1971, 21:1560–1562
9. Hadjiolov D, Frank N, Schmähl D: Inhibition of diethylnitrosmanine-induced strand breaks in liver DNA by disulfiram. Z Krebsforsch 1977, 90:107–109
10. den Tonkelaar EM, van Logten MJ: Protective action of dithiocarbamates on experimental liver dâmage produced by carbon tetrachloride. Toxicol Appl Pharmacol 1974, 30:96–106
11. Popp JA, Shinozuka H, Farber E: The protective effects of diethyldithiocarbamate and cycloheximide on the multiple hepatic lesions induced by carbon tetrachloride in the rat. Toxicol Appl Pharmacol 1978, 45:549–564
12. Solt DB, Medline A, Farber E: Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. Am J Pathol 1977, 88:595–618
13. Bergmeyer HU, Brent E: Glutamate-pyruvate transaminase, Methods of Enzymatic Analysis. Edited by HU Bergmeyer. New York, Academic Press, 1974, pp 752–767
14. Gerlach U, Hiby W: Sorbitol dehydrogenase, Methods of Enzymatic Analysis. Edited by HU Bergmeyer. New York, Academic Press, 1974, pp 569–573
15. Nachtomi E, Sarma DSR: Repair of rat liver DNA in vivo damaged by ethylene dibromide. Biochem Pharmacol 1977, 26:1941–1945
16. Marmur J: A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 1961, 3:208–218
17. Kirby KS, Cook EA: Isolation of deoxyribonucleic acid from mammalian tissues. Biochem J 1967, 104:254–257
18. Brookes P, Lawley PD: The alkylation of guanosine and guanylic acid. J Chem Soc 1961, pp 3923–3928

19. Singer B: Reaction of guanosine with ethylating agents. Biochemistry 1972, 11:3939–3947
20. Magee PN, Barnes JM: Carcinogenic nitroso compounds. Adv Cancer Res 1967, 10:163–246
21. Magee PN, Farber E: Toxic liver injury and carcinogenesis, Methylation of rat-liver nucleic acids by dimethylnitrosamine in vivo. Biochem J 1962, 83:114–124
22. Heath DF: The decomposition and toxicity of dialkylnitrosamines in rats. Biochem J 1962, 85:72–91
23. Magee PN, Lee KY: Cellular injury and carcinogenesis: Alkylation of ribonucleic acid of rat liver by diethylnitrosamine and n-butylmethyl-nitrosamine in vivo. Biochem J 1964, 91:35–42
24. Fiume L, Campadelli-Fiume G, Magee PN, Holsman J: Cellular injury and carcinogenesis: Inhibition of metabolism of dimethylnitrosamine by aminoacetonitrile. Biochem J 1970, 120:601–605
25. Stripp B, Greene FE, Gillette JR: Disulfiram impairment of drug metabolism by rat liver microsomes. J Pharmacol Exp Ther 1969, 170:347–354
26. Lutz LM, Glende EA Jr, Recknagel RO: Protection by diethyldithiocarbamate against carbon tetrachloride lethality in rats and against carbon tetrachloride induced lipid peroxidation in vitro. Biochem Pharmacol 1973, 22:1729–1734
27. Craddock VM, Magee PN: Reaction of the carcinogen dimethylnitrosamine with nucleic acids in vivo. Biochem J 1963, 89:32–37
28. Swann PF, Magee PN: Nitrosamine-induced carcinogenesis: The alkylation of nucleic acids of the rat by N-methyl-N-nitrosourea, dimethylnitrosamine, dimethyl sulphate and methyl methanesulphonate. Biochem J 1968, 110:39–47
29. Pegg AE: Alkylation of rat liver DNA by dimethylnitrosamine: Effect of dosage on O^6-methylguanine levels. J Natl Cancer Inst 1977, 58:681–687
30. Thiers RE, Reynolds ES, Vallee BL: The effect of carbon tetrachloride poisoning on subcellular metal distribution in rat liver. J Biol Chem 1960, 235:2130–2133
31. Reynolds ES, Thiers RE, Vallee BL: Mitochondrial function and metal content in carbon tetrachloride poisoning. J Biol Chem 1962, 237:3546–3551
32. Farber JL, El-Mofty SK: The biochemical pathology of liver cell necrosis. Am J Pathol 1975, 81:237–250
33. Chien KR, Abrams J, Pfau RG, Farber JL: Prevention by chlorpromazine of ischemic liver cell death. Am J Pathol 1977, 88:539–558
34. Izutsu KT, Smuckler EA: Effects of carbon tetrachloride on rat liver plasmalemmal calcium adenosine triphosphatase. Am J Pathol 1978, 90:145–158
35. Gallagher CH, Gupta DN, Judah JD, Rees KR: Biochemical changes in liver in acute thioacetamide intoxication. J Pathol Bacteriol 1956, 72:193–201
36. Calvert DN, Brody TM: Biochemical alterations of liver function by the halogenated hydrocarbons: I. In vitro and in vivo changes and their modifications by ethylene diamine tetraacetate. J Pharmacol Exp Therap 1958, 124:273–281
37. Schanne FAX, Kane AB, Young EE, Farber JL: Calcium dependence of toxic cell death: A final common pathway. Science (In press)

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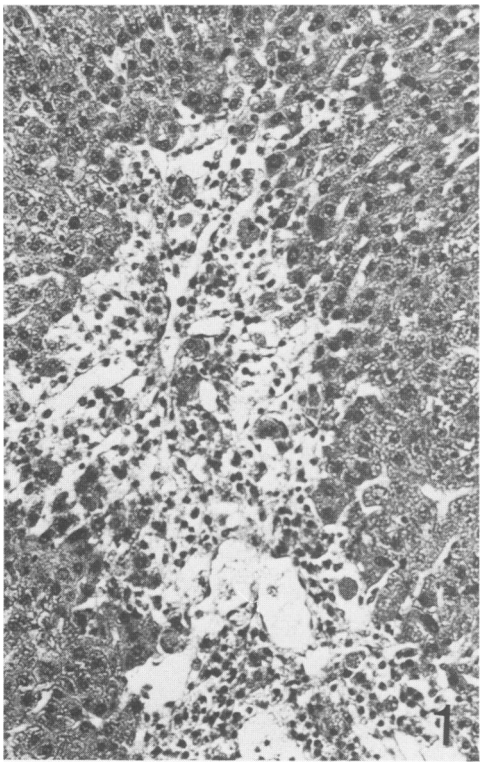
[*Illustrations follow*]

Figure 1—Photomicrograph of a section of liver from a rat treated with 100 mg/kg DEN and examined 3 days later. Necrosis and infiltration of inflammatory cells are seen in Zone 3. (H&E, ×100)

Figure 2—Photomicrograph of a liver section from a rat given DEDTC (3 × 50 mg/kg at 4-hour intervals) beginning 4 hours after the administration of 100 mg/kg DEN and examined 3 days later. Zone 3 cells show a moderate eosinophilic reaction and depletion of glycogen. Necrosis is minimal, except for a few isolated single cell deaths. (H&E, ×100)

Figure 3—Photomicrograph showing necrosis of the liver of a rat 3 days after intraperitoneal administration of 20 mg/kg DMN. (H&E, ×100)

Figure 4—Photomicrograph showing protection against DMN-induced hepatic necrosis by DEDTC. DEDTC (50 mg/kg) was given 3 times at 4-hour intervals commencing 4 hours after administration of 20 mg/kg DMN, and the liver was examined 3 days later. (H&E, ×100)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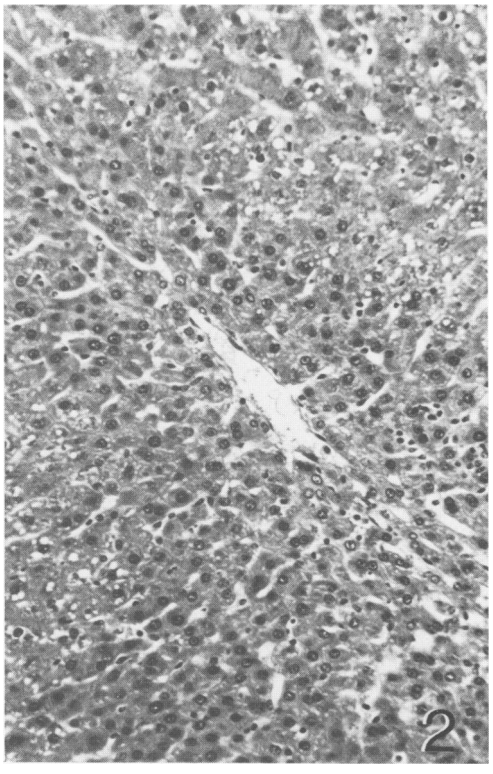
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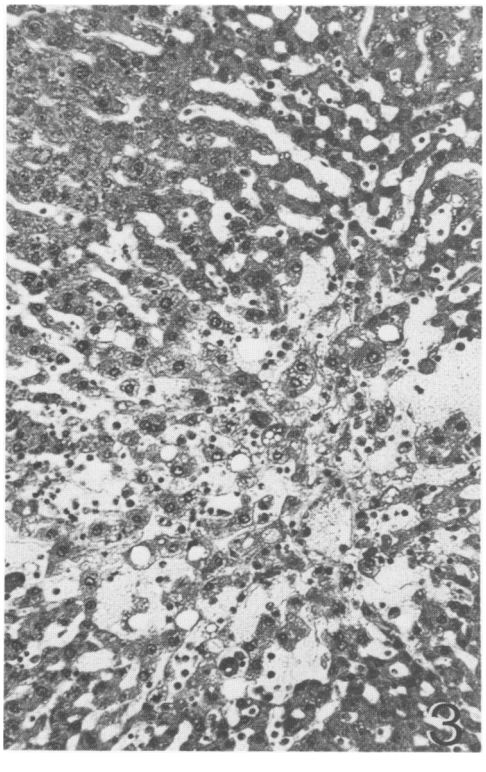
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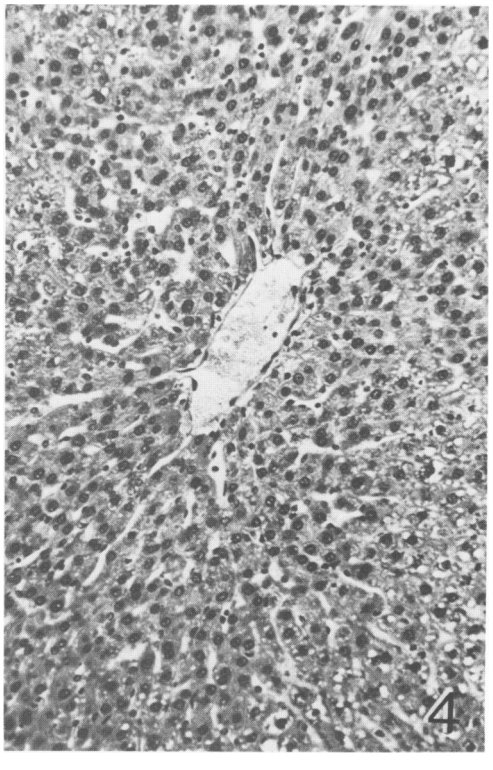
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