1,1-Dichloroethylene Hepatotoxicity

Time Course of GSH Changes and Biochemical Aberrations

Edward S. Reynolds, MD, Mary Treinen Moslen, MS, Paul J. Boor, MD, and Rudolph J. Jaeger, PhD

Exposure of fasted rats to 200 ppm 1,1-dichloroethylene (1,1-DCE) for 1–4 hours resulted in striking aberrations in hepatic Na, K, Ca, and GSH levels which preceded and/or accompanied catastrophic histologic alterations of the liver. Na levels began to rise during the first hour, and preceded the morphologically apparent injury. Ca levels increased markedly and K levels declined between the second and fourth hour of exposure, and accompanied the catastrophic morphologic alterations. GSH levels were rapidly depleted but began to recover before the end of the exposure to 1,1-DCE. Functions of components of the mixed-function oxidase system of the liver endoplasmic reticulum were not appreciably affected early in the course of 1,1-DCE exposure; but after injury became massive, cytochrome P-450 and oxidative N-demethylase were deactivated. Thus effects on the functional components of the endoplasmic reticulum mixed-function oxidase system do not appear to be primary events in 1,1-DCE cytotoxicity. In contrast, there were progressive declines in mitochondrial K and marked imbalances in mitochondrial Na, Zn, and Mg preceding the massive influx of Ca into the cell, indicating that mitochondria are involved early in the evolution of injurious molecular events elicited by this potent hepatotoxin. (Am J Pathol 1980, 101:331–344)

1,1-DICHLOROETHYLENE (1,1-DCE) is an exquisite hepatotoxin. It is more potent, faster acting, and has a far more precipitous dose threshold for injury in the fasted rat than the classic hepatotoxin carbon tetrachloride (CCl_4).[1,2]

1,1-DCE produces a distinctive morphologic pattern of injury that preferentially involves mitochondria, spares endoplasmic reticulum, produces chromatin segregation within nuclei, and causes cell borders to retract forming lacunar spaces within hepatic cords.[3] Thrombosis ensues and necrosis is manifest within 4 hours after the onset of exposure. This pattern of cell injury differs markedly from that caused by CCl_4 and by other chloroethylenes, including vinyl chloride and trichloroethylene, each of which primarily involves endoplasmic reticulum in a more gradually evolving process.[4]

From the Chemical Pathology Laboratory, Department of Pathology, University of Texas Medical Branch, Galveston, Texas, and the Kresge Center for Environmental Health, Harvard School of Public Health, Boston, Massachusetts. Dr. Jaeger's present address is Institute of Environmental Medicine, New York University Medical Center, New York, New York.

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Address reprint requests to Edward S. Reynolds, MD, Department of Pathology, University of Texas Medical Branch, Galveston, TX 77550.

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Techniques of Analysis

Hepatic injury was assessed by light-microscopic study, by the determination of Na, K, Mg, Ca, Zn, and Fe contents of wet-ashed liver samples with atomic absorption spectrophotometry,[10] and by measurement of the activities of three liver-derived enzymes in serum. The enzyme activities measured were serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) (Sigma Reagent Kits) and serum sorbital dehydrogenase (SDH).[11]

The frequency of mitotic figures were estimated in 4-μ paraffin sections according to the techniques of Weibel and Elias.[12]

The functional integrity of the isolated endoplasmic reticulum fraction was assessed by the assay of microsomal enzymes, primarily mixed-function oxidase components, according to Moslen et al,[13] and expressed in terms of microsomal protein content. The protein content was determined by the method of Lowry et al.[14]

We estimated GSH levels by measuring nonprotein sulfhydryl contents using Ellman's reagent according to Jaeger et al.[8] Metal and GSH levels of tissue fractions from individual animals were expressed in terms of the protein content.

Statistical Procedures

Care was taken in this time course study to compare "experimental" quantitative values obtained from the animals exposed to 1,1-DCE to appropriate "time of day control" values obtained from the animals exposed to air only and killed at compatible intervals during the experimental period. The quantitative values obtained from the animals exposed to air only remained relatively constant throughout the experimental period. Significant differences in t test comparisons were interpreted according to Fisher and Yates.[15] Changes were considered statistically significant if $P < 0.05$. Regression correlations were obtained by least-squares analysis with the use of a Wang Programmable Computer.

Results

Parameters of Injury

Histologic findings are presented in Figure 1. The most striking early histologic changes involved the nuclei. At 1 hour there was a threefold increase in the number of mitotic figures in parenchymal cells, as compared with their infrequent appearance in the hepatocytes of control rats (685 ± 146 vs 215 ± 23 mitotic figures/cu mm; or approximately 4.0 per 1000 hepatocytes vs 1.3 per 1000 hepatocytes); this increase is statistically significant ($P < 0.05$, $df = 6$). By 2 hours mitotic figures had all but disappeared, and parenchymal cells in centrolobular and midzonal areas began to show central rarefaction of nuclei with peripheral displacement of chromatin to nuclear margins. Concomitant with changes in chromatin distribution, cell borders of affected parenchymal cells were retracted, and pericellular lacunae formed within hepatic cords. Histologic injury was progressive, and by 4 hours frank hemorrhagic centrolobular necrosis was present. At subsequent time points of 6 and 12 hours (not presented in Figure 1) the

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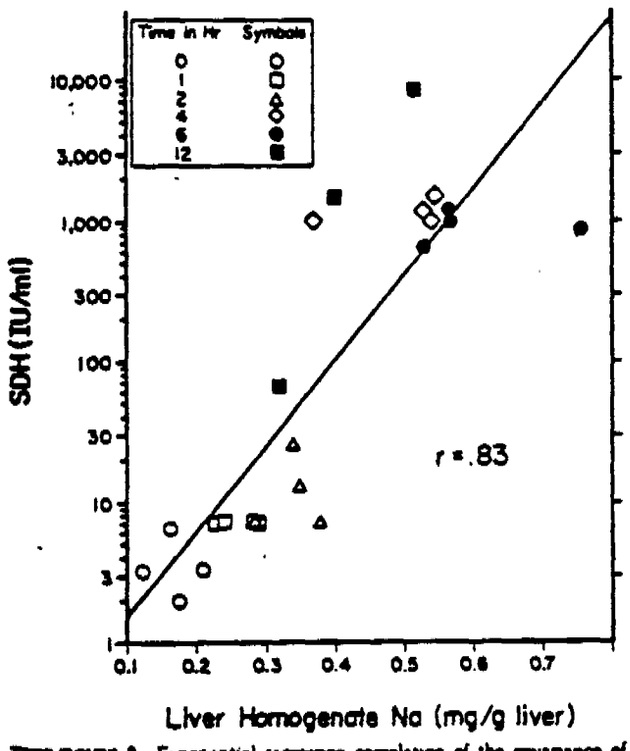
massive histologic injury was similar in character and extent to that observed at 4 hours.

Serum SDH activity became elevated and Na levels in liver increased by the end of the first hour of 1,1-DCE exposure. As indicated in Text-figure 1 (top and center) changes in these two parameters progressed during the second hour, while other serum enzymes and liver metals assayed were not appreciably altered. By the fourth hour, however, serum activities of the two transaminases, SGOT and SGPT, were elevated and liver Ca was increased markedly, while K, Mg, and Zn levels had decreased significantly below the values of control animals. Liver K, Mg, and Zn levels plateaued at below normal levels after the fourth hour, while Ca continued to accumulate through the twelfth hour. Liver Na peaked at 6 hours.

In order to determine how the changes in liver metal levels correspond



TEXT-FIGURE 2—Exponential regression correlation of the covariance of liver homogenate Na levels and serum SDH activities of individual 1,1-DCE-exposed animals killed during the 12-hour experimental period. Three to 4 animals were killed at each time point. Note the clustering of adjacent time points. The correlation is significant at $P \ll .001$ level ($F = 45.59$, $r = 0.83$, $df = 22$).

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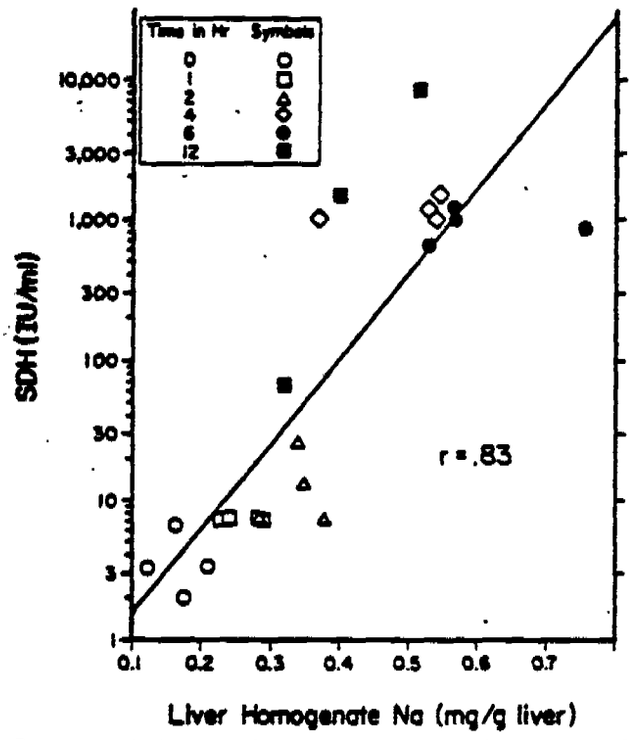

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ter panel). The GSH level at each time point from the first to the sixth hour after the onset of 1,1-DCE exposure is statistically distinct ($P < 0.025$) from that of the time before as well as the zero hour "control" point. Liver GSH contents were evidently replenished during the third and fourth hours of 1,1-DCE exposure, when the injury first became morphologically manifest.

Mitochondrial GSH levels abruptly decreased to approximately half control values during the first 2 hours of DCE exposure but, unlike total liver GSH, failed to rebound.

Mitochondrial Metals

Changes in mitochondrial Ca levels paralleled the abrupt increases in total liver calcium. Specifically, between 2 and 4 hours mitochondrial Ca increased 10-fold (Text-figure 1, lower panel). Mitochondrial K levels progressively decreased from two-thirds control values at 1 hour to one-eighth by 12 hours. Thus Ca displaced K as the dominant mitochondrial cation; the Ca/K mole ratio rose from 0.03 in controls to 1.9 at 12 hours after 1,1-DCE (Table 1). The mitochondrial Na levels decreased to half control levels by 6 hours, but not as steadily as total liver Na levels. The levels of the other metals fluctuated. The most striking fluctuation was for Zn, which decreased by more than half at 1 hour, and then partially recovered. Mg levels oscillated below control during 1,1-DCE exposure and then declined to approximately half normal. Mitochondrial Fe levels (not illustrated in Text-figure 1) steadily decreased, reaching one-fourth pre-exposure values by 12 hours. In contrast to the fluctuations in metal levels, protein contents of mitochondria (per gram wet weight) remained stable until 12 hours.

Endoplasmic Reticulum Function

The effects of 1,1-DCE exposure on microsomal enzyme components and activities were measured at 2 time points—2 hours, when injury is slight, and 6 hours, when injury is massive (Table 2). At 2 hours after the

Table 1—Effects of 1,1-DCE Exposure on the Ratio of Calcium to Potassium in Mitochondria

Time*	Moles Ca/moles K
0	0.03
1	0.04
2	0.05
4	0.70
6	1.74
12	1.92

* Hours after onset of 1,1-DCE exposure.

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ures. Since an abundant supply of GSH is reported to be conducive of fast and efficient mitosis,[16] the decrease in hepatocyte GSH could have contributed to an arrest of the normally brief (40–80 minutes) mitotic phase in cycling hepatocytes.[17] Alternatively, the influx of Na could have contributed to a rapid stimulation into mitosis of hepatocytes blocked in the late G_2 phase of the cell cycle. An increase in Na ion influx has been found critical to hepatocyte proliferation *in vitro*,[18] and rodent hepatocytes have been found to contain a subpopulation of G_2-blocked hepatocytes that can rapidly be stimulated into mitosis.[19]

The progressive Na influx during the first 2 hours of 1,1-DCE exposure should be considered as a potential contributing factor to the segregation and retraction of nuclear chromatin observed after 2 hours of 1,1-DCE exposure, since alterations in Na levels have been found to affect the organization and structure of chromatin preparations.[20,21] Of course, other biochemical changes, as yet undetermined, including activation of endonucleases and proteases, could contribute to the observed rapid conversion of hepatocyte chromatin from highly integrated nucleic acid–protein complexes to segregated pools of DNA, RNA, and protein.

Although this time course study indicates a close correspondence between the early Na influx and increases in the serum activities of sorbital dehydrogenase above its low background levels (Text-figure 1), appreciable alterations either in the level of K or Ca in the liver or in the serum activities of liver-derived transaminases were not detected at early times. While it is possible that a generalized "leakiness" of the plasma membrane accounts for the marked early entrance of Na, the lack of equivalent and concomitant changes in other soluble cytoplasmic components indicates a more specific effect.

One obvious kind of specific effect that could explain the early Na influx is a problem with membrane ion pumps, either directly or indirectly due to ATP deficiency. An indirect effect of 1,1-DCE on the ion pumps due solely to an ATP deficiency appears questionable, since ethionine administration, which rapidly and dramatically depletes liver ATP levels, causes not only a rapid increase in liver Na levels but also a nearly equivalent decrease in liver K levels.[22,23]

We did measure an early and progressive loss of K from the mitochondria following 1,1-DCE (Text-figure 1, lower panel). This loss of mitochondrial K is suggestive of a deficiency in metabolic energy, since accumulation of K by the mitochondria is an active process dependent on availability of metabolic energy.[24] The initial loss of K cannot be attributed to Ca, for loss of mitochondrial K precedes by several hours the influx of Ca into the cell. However, at later times the K loss may be aggra-

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first substrate on the enzyme was found to be the electrophilic one, and for very reactive substrates the substrate–enzyme reaction was irreversible, with the enzyme virtually "committing suicide". Therefore, we suggest that the catastrophic histologic and biochemical changes observed were a consequence of the continued formation of reactive 1,1-DCE species coupled with abatement of the capacity to detoxify such reactive species.

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