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Effect of 18 HR Fast and Glutathione Depletion on 1,1-Dichloroethylene-Induced Hepatotoxicity and Lethality in Rats[1,2]

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Four-hour inhalation exposure to 1,1-dichloroethylene (1,1-DCE, vinylidene chloride) was more injurious to 18-hr (overnight) fasted rats than to rats fed *ad libitum*. The estimated 24 hr LC50 for fed rats was 15,000 ppm while the same value for fasted rats was 600 ppm. The minimum lethal concentration was 200 ppm for fasted rats and 10,000 ppm for fed rats. Serum alanine α-ketoglutarate transaminase (AKT) elevation occurred at 150 ppm in fasted rats, but in the fed rats, a significant elevation was only seen at 2000 ppm and higher. Elevated serum AKT preceded hepatic necrosis and death. This fed-fasted difference in serum AKT elevation was also demonstrable in an isolated perfused rat liver system. The AKT elevation in perfusate from livers of fasted rats was consistent with the time course of injury seen *in vivo*. Increased susceptibility to hepatic injury appeared to be related to decreased hepatic glutathione concentration associated with fasting (18 hour). Diethylmaleate, a material which results in a decreased hepatic glutathione concentration was administered *in vivo* and *in vitro*. This treatment potentiated the hepatic injury in fed rats and in livers taken from fed rats and subsequently perfused.

INTRODUCTION

The chlorinated hydrocarbon, 1,1-dichloroethylene (1,1-DCE)[3] is used in the manufacture of Saran type plastics, and has been reported to be a contaminant of submarine and spacecraft atmospheres (Altman and Dittmer, 1966). Recently Kramer and Mutchler (1972) reported that workers in polyvinyl chloride manufacturing plants have a slight (approximately 5 ppm) exposure to 1,1-DCE. A time-weighted dose-response relationship for some indices of hepatic injury was shown for work-related vinyl chloride/vinylidene chloride exposure.

The studies to be reported here are an extension of previous studies on the biochemical effects and mechanism of hepatotoxic action of 1,1-DCE (Jaeger *et al.*, 1973; Jenkins *et al.*, 1972). Our earlier studies utilized oral doses of 1,1-DCE in fasted animals while inhalation exposures of both fed and fasted

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[3] 1,1-Dichloroethylene is also referred to as vinylidene chloride or 1,1-dichloroethene.

rats were used in the present investigation. A preliminary communication was made (Jaeger *et al.*, 1973a) in which fasted rats were found to be more sensitive to the lethal and hepatotoxic effects of 1,1-DCE. The present report describes studies on the mechanism of this enhancement of toxicity.

MATERIALS AND METHODS

Animals and Treatments

Male Holtzman rats weighing between 250 and 400 g were used. They were kept in an air conditioned room on a daily 12 hr light cycle (6 AM–6 PM) and were supplied with Purina chow and water *ad libitum*. The animals were fasted from 4 PM on the day prior to exposure. All animals were allowed free access to water during the fasting period, except that neither food nor water was available during the period of exposure to 1,1-DCE. After exposure fed rats were again given food. The animals were sacrificed by cervical transsection and exsanguination.

In other experiments animals were pretreated with diethylmaleate (DEM). The diethylmaleate was prepared as a 50% solution in propylene glycol and administered intraperitoneally. Control animals received equal volumes of the diluent.

Inhalation Exposure

Exposures were performed from approximately 10 AM to 2 PM in a 30 liter dynamic-flow chamber described by Leach (1963). Five to 10 animals were placed in the chamber with an air flow of 15–20 liters per minute. 1,1-DCE vapor was generated using an air metering system and fritted glass vaporizers which were kept in a constant temperature bath to allow adjustment of ambient water temperature. This was necessary since the boiling point of 1,1-DCE is 32° C and precise temperature control was required to achieve concentrations in the range of 50 to 20,000 ppm.

Concentrations of 1,1-DCE in the exposure chamber were determined with a Varian 1700 gas–liquid chromatograph (GLC). Approximately 30 ml air samples were taken from the chamber and injected into the gas chromatograph using a gas sampling valve. The valve (1.19 ml volume) permitted highly reproducible sampling of the gas mixture. Detection of the 1,1-DCE vapor was with a hydrogen flame ionization detector. The GLC was standardized with .1, .5 or 1% solutions of 1,1-DCE in hexane. The gas chromatographic conditions were as follows: injector temperature: 100° C, oven temperature: 160° C, detector temperature: 160° C, carrier gas-nitrogen at 30 lbs pressure, Column: Poropak Q 80–100 mesh in a 1/8 inch stainless steel column. Calculations of chamber 1,1-DCE concentrations were made at 15–30 min intervals using a constant derived from the peak heights of the standards. Adjustments of 1,1-DCE vapor flow into the chambers were made as frequently as necessary to maintain a stable vapor concentration during the four hour exposure. In general, variations of 1,1-DCE concentration did not exceed 10% of the stated time-weighted mean chamber concentrations.

Isolated Perfused Liver

The isolated perfused liver system as described by Ruderman *et al.*, (1967) and Miller (1961) was used. The perfusion medium was prepared as follows. A concentrated solution of bovine serum albumin (BSA) in Kreb's ringer bicarbonate buffer (KRB) was dialyzed in two changes of KRB. The BSA solution was made to 4% with additional KRB and 5 mg% each of penicillin and streptomycin were added to prevent bacterial growth. Two perfusate compositions were used in this study. One, a perfusate approximating the plasma composition from fed animals contained KRB, 4% BSA, penicillin, streptomycin and 360 mg% glucose. A second perfusion medium contained KRB, 4% BSA, penicillin, streptomycin, 100 mg% glucose, 10 m*M* alanine and 1 μmole/ml sodium oleate. During perfusions with this latter medium, sodium oleate was added to the perfusate at the rate of 200 μmoles/hr. The composition of the perfusate had no effect on the hepatotoxic response, and all data from these experiments were pooled. The total perfusate volume was 100 ml.

Fed or fasted rats were anesthetized with pentobarbital sodium (50 mg/kg). A midline incision was made, the bile duct was canulated with PE-10 tubing and the portal vein was canulated using PE-240 gauge tubing. The liver was carefully removed from the animal, and connected to the perfusion apparatus in a heated cabinet. Temperature in the perfusion cabinet was maintained at 37.5° and the liver perfusate was exposed to a gas-phase of humidified 95% oxygen, 5% CO_2. Vapors of 1,1-DCE were added to this gas phase using a dynamic vaporizing system as described for the inhalation exposure. 1,1-DCE concentration in the gaseous phase was determined by GLC techniques as described above.

The livers, weighing from 7–9 g (fasted rats) and 11–14 g (fed rats), were perfused for 3 hr. Perfusate flow rates at the beginning of the experiments were between 100 and 150 ml/min/liver. Flow was measured using a calibrated outflow tube with a volume of 5 ml. The livers were discarded if, after an initial 15 min perfusion interval, the total flow through the liver was not at least 50 ml/min. Although bile flow was established, the flow rate was not measured. Samples of the perfusate (2 ml) were taken at 30 min intervals. In some experiments diethyl maleate (25 μl) was added to the perfusion medium with a Hamilton microsyringe.

At the end of the perfusion the canulae and extraneous tissue were removed; the livers rinsed, blotted and weighed. Livers and perfusate samples, as well as sera from *in vivo* exposures were kept frozen until assayed.

Biochemical Assays

Serum or perfusate alanine α-ketoglutarate transaminase (AKT) was measured by the method of Murphy and Malley (1969). Nonprotein sulfhydryl concentration, expressed as glutathione, was measured as follows: a 20% liver homogenate was made in 5% TCA-5 m*M* Na_2EDTA using a polytron homogenizer. The homogenate was centrifuged and 0.4 ml of the supernatant was added to 4.5 ml of ph 8 phosphate buffer (0.1 *M*). Fifty microliters of Ellman reagent (1959) was added and the optical density was measured at 412 nm in a Gilford 240 spectrophotometer. Concentration of GSH/g liver was determined using authentic GSH standards.

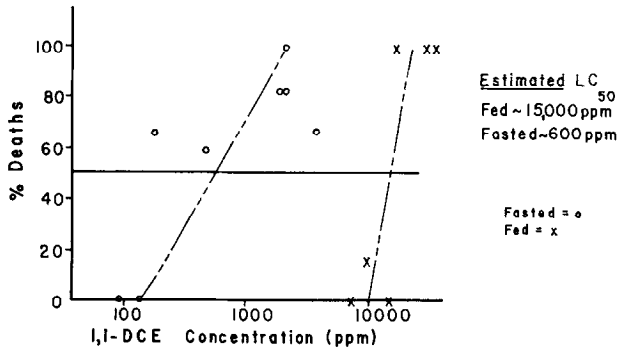

FIG. 1. Effect of fasting on the 24 hr mortality following a 4 hr 1,1-DCE inhalation exposure. Each point represents the percent of animals dead after exposure to 1,1-DCE with group sizes of 5 or 6. The broken line represents an approximation of the dose-response curve. The 1,1-DCE concentration is plotted logarithmically.

Statistics

Significance of differences between means was tested with the Student's t test or the Mann–Whitney test using a 2 tailed level of significance equal to $P \leq 0.05$.

RESULTS

Inhalation Studies in Vivo

The effect of feeding history on the 24 hr mortality after 1,1-DCE vapor exposure is shown in Fig. 1. The animals used in this experiment were divided into 2 groups. One group was fasted for 18 hours (4 PM–10AM) prior to exposure and the other group was supplied with food *ad libitum*. The estimated 24-hr LC50 following a 4 hr exposure for fasted rats was 600 ppm. The fed rats

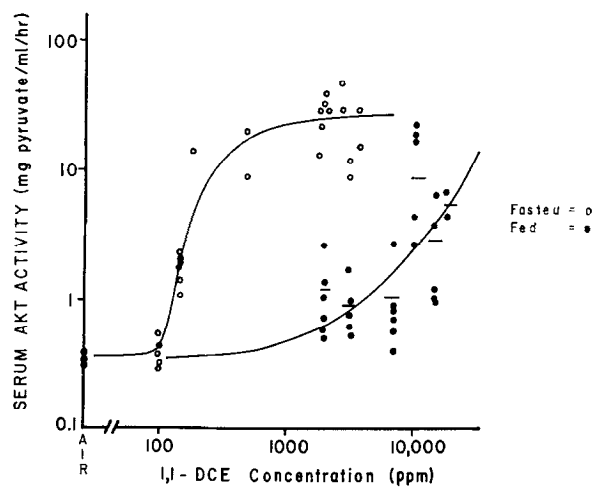

FIG. 2. Effect of fasting on the 24 hr serum AKT elevation after a 4 hr 1,1-DCE inhalation exposure. Each point represents the mean of duplicate serum AKT determinations for each animal. The data are plotted logarithmically. The solid lines are approximations to the line of best fit using the group average value represented by a dash. Only 24 hr survivors were tested.

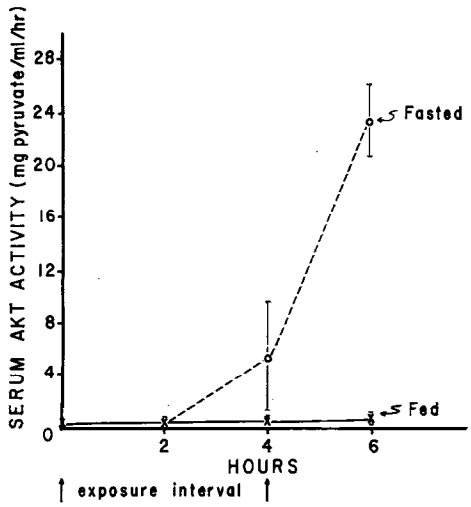

FIG. 3. Time course of 1,1-DCE induced elevation of serum AKT in fed and fasted rats. The animals were sacrificed at the times specified. Control serum AKT activity was approximately 0.3 mg pyruvate/ml/hr. The concentration of 1,1-DCE was 2000 ppm. The data for fasted rats at 4 hr was not significantly different by statistical test at $P \leq 0.05$ level from the fed rats (4 hr serum AKT range for fed animals—0.42–0.53 vs fasted animals—0.52–17.76). The 6 hr point was significant ($P < 0.05$), however. Four rats were used at each time point.

were much more resistant to the lethal action of inhaled 1,1-DCE, with an estimated LC50 of 15,000 ppm. Although the data for the fasted rats are somewhat variable, the minimum lethal concentration in the fasted rat was 200 ppm while this value was 10,000 ppm in the fed rat.

The effect of fasting on elevation of serum AKT activity after 1,1-DCE vapor exposure was determined. Earlier work in our laboratory (Jaeger *et al.*, 1973, Jenkins *et al.*, 1972) showed this enzyme to be a useful one in assessing hepatotoxicity after 1,1-DCE. The data in Fig. 2 are derived from those animals which survived for 24 hr after exposure. The lines shown in this figure were drawn using the mean values obtained from each exposure concentration. It can be seen that fasted rats were more sensitive to the hepatotoxic action of 1,1-DCE. A significant ($P < 0.05$) elevation of serum transminase activity at 24 hours was noted in fasted rats exposed to as little as 150 ppm of 1,1-DCE. The apparent plateau in serum AKT elevation of the fasted rats may reflect the fact that the more severely injured rats died.

In Figure 3 the effect of exposure to 2000 ppm 1,1,-DCE on the time course of serum AKT activity in fed and fasted rats is shown. Rats were removed and sacrificed after 2 or 4 hr of continuous exposure to 1,1-DCE or after 4 hr exposure to 1,1-DCE followed by 2 hr of air. It can be seen that serum AKT activity increased quite rapidly in fasted rats. No increase in serum AKT activity was observed in fed rats exposed under the same conditions.

An experiment was performed to determine if the fed-fasted difference in 1,1-DCE sensitivity could be reduced by refeeding a fasted animal or fasting a fed animal. Fed rats were deprived of food from 9 AM and fasted rats were allowed access to food from 9 AM following an overnight fast. Exposure to

TABLE I

EFFECT OF REFEEDING OR FASTING ON 1, 1-DCE[a]-INDUCED HEPATOTOXICITY

	Feeding Schedule Day 1 4 PM–9 AM	Day 2 1 PM–10 AM	SAKT[b] Activity of 24-hr Survivors (N)
I	fed	fed	1.07 ± 0.37 (6)[c]
II	fee	fast	0.86 ± 0.20 (5)[c]
III	fast	fast	30.88 ± 3.21 (4)[d]
IV	fast	fed	33.30 ± 3.97 (3)[d,e]

[a] 2000 ppm 1, 1-DCE × 4 hr.
[b] Control activity = 0.27 ± 0.02 mg pyruvate/ml serum/hr.
[c] $P < 0.05$, I & II compared to controls.
[d] $P < 0.01$, III & IV compared to I & II.
[e] $P > 0.05$, IV compared to III.

1,1-DCE was begun at 11 AM and continued til 3 PM; the surviving rats were sacrificed 24 hr later. The results of this experiment, shown in Table I, demonstrate that fasting a fed animal or permitting a fasted animal access to food has no effect on the subsequent production of liver injury as reflected by serum AKT elevation.

To determine if the increased sensitivity of the fasted rat was due to altered distribution of 1,1-DCE, two groups of rats (fasted or fed) were exposed to approximately 2000 ppm 1,1-DCE. After 2 hr of exposure, the animals were removed from the chamber, sacrificed, hexane extracts made of blood and liver, and 1,1-DCE concentration determined gas chromatographically. The results are shown in Table II. It can be seen that the blood concentration of 1,1-DCE was slightly higher in fasted rats. Similarly, when expressed in terms of micrograms 1,1-DCE per gram of liver, the fasted rats had a non-significant but slightly higher 1,1-DCE concentration. When correction was made for total liver size, 1,1-DCE concentration was similar between fed and fasted animals. Thus, while a preferential absorption of 1,1-DCE from the lung by blood may occur with a resultant slightly higher liver concentration in the fasted rat, the total amount of 1,1-DCE in the liver of fasted rats was not in excess of that found in the fed rat. Preferential distribution cannot account, therefore, for the observed difference in sensitivity.

TABLE II

BLOOD AND LIVER 1, 1-DCE CONCENTRATION IN FED AND FASTED RATES AFTER A 2-HR EXPOSURE[a]

Feeding History (N)	Blood μg/ml	Liver μg/g	μg/liver equivalent to 100 g body weight[b]
Fasted (3)	79.6 ± 9.8	34.9 ± 1.7	99.9 ± 6.4
Fed (3)	54.7 ± 1.9[c]	30.6 ± 2.0[d]	120.0 ± 6.8[d]

[a] 2 hr at 2275 ppm 1, 1-DCE.
[b] Corrected for decreased liver size due to fasting.
[c] $P < 0.05$ when compared to concentration in blood from fasted rats.
[d] $P > 0.05$ when compared to fasted rat liver concentration.

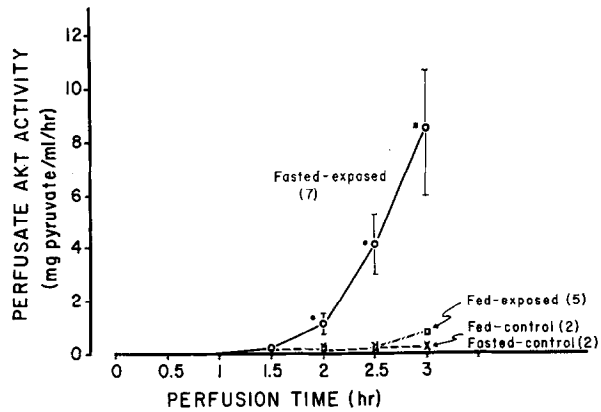

FIG. 4. Effect of 1,1-DCE exposure during perfusion on perfusate AKT elevation in livers from fed and fasted rats. Perfusate AKT activities were significantly ($P \leq 0.05$) elevated at 2, 2.5 and 3 hr relative to controls or livers from fed rats. At 3 hr, while perfused livers from fed-exposed rats demonstrated a slight elevation in perfusate AKT, the elevation was not significant ($P > 0.05$). The number in parenthesis is the number of perfused livers tested.

In Vitro Perfusion Experiments

In order to determine if 1,1-DCE-induced hepatic injury results from a direct toxic effect on the liver, a series of perfusion experiments were undertaken. The hypothesis that 1,1-DCE-induced liver injury might be demonstrated in a 3 hr perfusion of isolated livers from fasted rats was tested. Such a possibility was suggested by the time course of serum AKT activity shown in Fig. 3. The data for this series of experiments are shown in Fig. 4. 1,1-DCE at a concentration of 20,000 ppm in the gas phase was used. Control livers were perfused in absence of 1,1-DCE and after 3 hr of perfusion, no perfusate AKT elevation was seen. Of five 1,1-DCE-exposed livers from fed rats, 4 livers were similar to control, i.e., at the end of 3 hr of perfusion, there was no increase in liver weight or perfusate AKT elevation. With one fed-liver there was a significant elevation in perfusate AKT at the 3 hr time point. In this one experiment, however, no significant increase in liver weight had occurred by 3 hr. In contrast, the data from seven 1,1-DCE-exposed perfused livers from fasted rats are also shown in this figure. These livers began to appear grossly damaged by 2 hr. The livers became pale, swollen, and flow decreased. At the end of perfusion (3 hr) their weight had increased markedly, a result suggesting edema. As the data show, a significant ($P < 0.05$) perfusate AKT increase occurred by 2 hr, and this continued to increase thereafter. The increased perfusate AKT correlated well with appearance of grossly observable injury.

In an attempt to determine a biochemical difference between animals in the fed or fasted state which could account for the enhanced sensitivity of the fasted rat, several factors were considered. As noted in Methods, the inclusion of alanine and sodium oleate, a free fatty acid, in the perfusate, a condition approximating fasting plasma, did not increase the susceptibility to 1,1-DCE of livers from fed rats. Neither did high glucose concentration (360 mg%) confer protection on livers from fasted animals. In other experiments cold stress and epinephrine did not result in increased sensitivity in fed animals subsequently exposed *in vivo* to 1,1-DCE.

TABLE III

EFFECT OF 18 HR FAST ON HEPATIC GSH AND SAKT FOLLOWING 1, 1-DCE EXPOSURE (1000 PPM × 4 HR)

	Treatment (N)		Liver GSH Concentration[a] mg/g	Serum AKT Activity mg pyruvate/ml serum/hr
I	fed-air control	(5)	1.75 ± 0.08	0.27 ± 0.02
II	fasted-air control	(5)	1.50 ± 0.06[b]	0.19 ± 0.02
III	fed-1, 1-DCE exposed	(5)	0.89 ± 0.06[c]	0.62 ± 0.12[e]
IV	fasted-1, 1-DCE exposed	(5)	0.59 ± 0.07[d]	17.55 ± 4.13[f]

[a] Total liver GSH in controls (I & II): 18.82 ± 1.07 mg, fed rats vs. 11.78 ± 0.62 mg, fasted rats.
[b] $P < 0.05$, II vs. I.
[c] $P < 0.05$, III vs. I.
[d] $P < 0.05$, IV vs. II.
[e] $P < 0.05$, III vs. I or II.
[f] $P < 0.05$, IV vs. I, II or III.

Glutathione Depletion In Vivo and In Vitro

Recently, work by Brodie *et al.*, (1971) and by Reid and Krishna (1973) has suggested a role of glutathione (GSH) depletion in the development of centrolobular necrosis following bromobenzene intoxication. Since 1,1-DCE also caused central necrosis, it was suspected that differences in hepatic glutathione concentrations between the fed and fasted state would account for differential sensitivity to 1,1-DCE. The observation of GSH difference with fasting was reported by Maruyama, *et al.* (1968). Hepatic GSH concentration was measured and a significant difference was found between fed and fasted animals that were sacrificed at 10 AM, the time when 1,1-DCE exposure was normally begun. The data, shown in Table III, demonstrate that GSH on a mg/g basis as well as total mg GSH is lower in the livers of fasted rats than fed rats. Exposure to 1,1-DCE vapor caused a reduction in hepatic GSH in both fed and fasted rats. However, the degree of reduction did not correlate with hepatic injury. In this case, fed rats had a 2-fold elevation of serum AKT suggesting slight hepatotoxicity while fasted rats had almost a 100-fold elevation of this enzyme.

In order to test the hypothesis that GSH depletion causes enhanced sensitivity to 1,1-DCE, an experiment was conducted in which fed rats were pretreated with diethylmaleate (Boyland and Chasseaud, 1970). The dose employed (0.25 ml/kg, ip) was sufficient to cause a greater than 50% reduction in GSH concentration at 30 min after dosing (R. Richardson, this laboratory, unpublished observation). Thus, fed animals could be depleted of GSH and rendered simliar to fasted animals, at least in this respect. The data from this experiment (Table IV) involved two groups of rats: one group was pretreated with DEM in propylene glycol (50%), and the other group was treated with equal amounts of the vehicle. Thirty minutes after dosing, 5 rats of each group were exposed to 1000 ppm of 1,1-DCE for 4 hr. The data show that DEM did cause a 62% reduction in GSH at 30 min after dosing (0 hr), and GSH was still 29% depleted by 6 hr. At 6 hr after the start of 1,1-DCE exposure, vehicle treated animals exposed to 1,1-DCE had a significant increase in serum AKT (3.8 fold).

TABLE IV

EFFECT OF DIETHYLMALEATE (DEM) (0.25 ML/KG) PRETREATMENT ON HEPATIC GSH AND SAKT FOLLOWING 1, 1-DCE EXPOSURE (1000 PPM × 4 HR) IN FED RATS

	Pretreatment	N	Time hr	Exposure	Hepatic GSH mg/g	Serum AKT mg/pyruvate/ml/hr (range)
I	Propylene glycol	(3)	0	Air	2.18 ± 0.12	—
II	Propylene glycol	(3)	6	Air	2.01 ± 0.17	0.32 ± 0.06 (0.24 – 0.43)
III	DEM & Propylene glycol	(3)	0	Air	0.84 ± 0.14	—
IV	DEM & Propylene glycol	(3)	6	Air	1.42 ± 0.18	0.47 ± 0.04 (0.43 – 0.54)
V	Propylene glycol	(5)	6	1, 1-DCE	1.02 ± 0.08	1.23 ± 0.13 (0.84 – 1.56)
VI	DEM & Propylene glycol	(5)	6[a]	1, 1-DCE	0.82 ± 0.13	19.48 ± 9.57[b,c] (1.42 – 56.14)

[a] One animal in this group died at 5 hr.
[b] $P = 0.05$, VI vs. V (Mann–Whitney test).
[c] $P < 0.05$, VI vs. IV (Mann–Whitney test).

Hepatic GSH in vehicle treated rats was also reduced (51% of control) by 1,1-DCE exposure. DEM pretreated animals had a similar depletion of GSH but serum AKT was markedly elevated (42 fold). The data shown in brackets indicate the range of serum AKT that was measured in each case. One of the 5 DEM pretreated, 1,1-DCE exposed animals did not differ in serum AKT

TABLE V

EFFECT OF DIETHYLMALEATE (25 μl) ON PERFUSATE AKT USING LIVERS FROM FED RATS THAT WERE EXPOSED TO 1, 1-DCE VAPOR DURING A 3 HR PERFUSION

Treatment (N)	Time 0. hr	0.5 hr	1.0 hr	1.5 hr	2.0 hr	2.5 hr	3.0 hr
Fed-DEM-Control (4)	0[a]	—	0.10[b] ±0.03	—	0.14[b] ±0.05	—	0.24[b] ±0.12
Fed-DEM-Exposed[c] (4)	0	0.06[b] ±0.02	0.09 ±0.02	0.15[b] ±0.04	0.66 ±0.24	3.14[b] ±1.05	8.0[d] ±2.13
Fasted-Exposed[c] (7	0	0.03 ±0.01	0.09 ±0.03	0.16 ±0.03	1.10 ±0.37	4.08 ±1.17	8.53[d] ±2.10
Fed-Exposed[c] (5)	0	0.02 ±0.01	0.05 ±0.01	0.08 ±0.02	0.12 ±0.02	0.19 ±0.05	0.79[e] ±0.37

[a] The perfusate AKT at 0 time was arbitrarily set at 0.
[b] All mean perfusate AKT values shown are in units of mg pyruvate/ml/hr ± the standard error of the mean.
[c] 1, 1-DCE concentration 20,000 ppm.
[d] $p < 0.05$ compared to fed-DEM-control.
[e] $p > 0.05$ compared to fed-control with or without added DEM.

activity from the vehicle treated, exposed rats. The remaining 4 DEM treated rats exposed to 1,1-DCE had signs of liver injury, i.e., bloody ascites and hemmorrhagic liver enlargement. One of these died at 5 hr, a mortality which was highly significant since it occurred at a concentration of 1,1-DCE well below the minimum lethal concentration for fed rats (see Figure 1 and text).

To further test the hypothesis that alteration of hepatic GSH *per se* is a modifier of hepatic injury after 1,1-DCE, the *in vitro* effect of DEM was tested in the isolated rat liver system. Livers from fed rats were perfused as described previously. DEM (25 μl) was added directly to the perfusate at time zero. Control livers were perfused without 1,1-DCE while experimental livers were exposed to 20,000 ppm as previously described. It should be noted that GSH depletion by acute doses of DEM is not known to cause liver injury or necrosis (J. R. Gillette, personal communication). The data from these experiments (Table V) show that DEM treatment of perfused livers from fed rats did not cause an elevation of perfusate AKT. Treatment with DEM and 1,1-DCE of perfused livers from fed rats resulted in an increase in perfusate AKT that was comparable in magnitude and time course to that seen in the perfused livers from fasted rats (data for perfusate AKT of fed and fasted livers from Fig. 4 reproduced in Table IV for comparison).

DISCUSSION

In this investigation we have shown that hepatotoxicity as measured by serum AKT elevation and lethality after 1,1-DCE inhalation are potentiated by overnight fasting of male rats. Protection against hepatic injury was not restored by refeeding fasted animals, nor was preferential uptake of 1,1-DCE by livers from fasted rats accountable for the enhancement of toxicity. Livers from fasted rats had somewhat lower GSH concentrations than fed rats. This decreased concentration could be related to the enhanced sensitivity of livers from fasted rats both *in vivo* and *in vitro*. This was confirmed in the intact liver and in the isolated perfused rat liver by the use of diethylmaleate, a compound which has been reported to deplete hepatic GSH (Boyland and Chasseaud, 1970). In both cases, normally insensitive livers from fed rats were rendered susceptible to 1,1-DCE toxicity after GSH depletion.

The mechanism of 1,1-DCE hepatotoxicity is not understood (Jenkins *et al.*, 1972: Carlson and Fuller, 1972). It is known to differ from a carbon tetrachloride-like mechanism (Jaeger *et al.*, 1973). In one respect, potentiation by DEM, 1,1-DCE appears to act by a mechanism similar to that proposed by Brodie *et al.* (1971) for bromobenzene. Bromobenzene is activated by inducible microsomal enzymes (Reid *et al.*, 1971). The proposed active metabolite, an epoxide, is a labile electrophillic alkylating agent. GSH acts as a site of loss (detoxification) while other tissue sulfhydryl groups are sites of attack (toxicity). Such attack is presumed to consist of covalent binding and subsequent liver injury, i.e., necrosis. Reid and Krishna (1973) have shown that diethylmaleate pretreatment enhances the necrotic action of bromobenzene. It should be noted that both 1,1-DCE and bromobenzene decrease hepatic glutathione concentration (this report, Tables III and IV: Varga, 1963).

The metabolic fate of 1,1-DCE is unknown. While epoxide formation may be proposed, there is no data currently available on the products of 1,1-DCE bio-

transformation. Further, the data of Brodie and co-workers require activation of bromobenzene since bromobenzene is normally relatively unreactive under physiological conditions. Reid and Krishna (1973) reported that bromobenzene-induced necrosis occurred 24 hr after dosing. 1,1-DCE, on the other hand, is a reactive species capable of forming polymers with itself and other materials following free radical initiation. In this respect activation by enzymes of the endoplasmic reticulum may not be necessary to produce injury. In this report, depletion of GSH, a soluble compound and a possible site of loss, could permit 1,1-DCE to act directly as an alkylating reagent on other tissue components, e.g., membranes. It should be noted that hepatic injury occurs quite rapidly after inhalation exposure, e.g. hemorrhage, and necrosis was observed in hematoxylin and eosin sections of liver within 2 hr of a 4 hr exposure (2000 ppm) in the fasted rat. Further, serum AKT elevation correlated well with the degree of observable morphologic damage (Jaeger and Averill, unpublished observation). The rapid time course of hepatic injury as reflected by serum AKT elevation (6 hr *in vivo* and 2 hr in the perfused liver) is consistent with a hypothesis based on direct tissue damage by 1,1-DCE without the need for prior activation. The work of Jenkins *et al.* (1972) suggested that microsomal enzyme induction by phenobarbital pretreatment was slightly protective after oral doses of 1,1-DCE. This suggests that 1,1-DCE itself is either the toxic species, or if activation occurs, it is not inducible by phenobarbital. Such a hypothesis is strengthened by the work of Carlson and Fuller (1972) who found that phenobarbital and methylcholanthrene induction did not further increase the elevation of serum transaminase activity after inhalation of 1440 ppm of 1,1-DCE when compared to the same exposure without pretreatment.

The enhancement of 1,1-DCE toxicity by overnight fasting, with its concomitant depletion of GSH is of importance for two reasons: first, from the standpoint of reproducibility in toxicity testing, and second, from an industrial hygiene point of view. We have also reported on the circadian periodicity of GSH and have shown fed animals to be more sensitive to the hepatotoxic and lethal actions of 1,1-DCE if exposures are conducted at night (10 PM–2 AM), a time when hepatic GSH concentrations are at or near their minima (Jaeger *et al.*, 1973b). If these findings apply to humans they would suggest enhanced susceptibility at times of low hepatic GSH concentration. The possibility of human exposure to 1,1-DCE was recently discussed by Kramer and Mutchler (1972). If GSH protects against hepatic damage, night shift or rotating shift work involving traces of 1,1-DCE could result in an enhanced possibility of work related injury. Such a suggestion is even more pertinent if other compounds are found to share a fed-fasted and day–night enhancement of toxicity such as described for 1,1-DCE.

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