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Potentiation of 1,1-Dichloroethylene Hepatotoxicity: Comparative Effects of Hyperthyroidism and Fasting[1]

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Potentiation of 1,1-Dichloroethylene Hepatotoxicity: Comparative Effects of Hyperthyroidism and Fasting. KANZ, M. F., WHITEHEAD, R. F., FERGUSON, A. E., AND MOSLEN, M. T. (1988). *Toxicol. Appl Pharmacol* **95**, 93–103. The responses of fed, fasted, and hyperthyroid (T_4) Sprague–Dawley male rats to 50 mg 1,1-dichloroethylene (1,1-DCE)/kg were compared. Hyperthyroid rats received three sc injections of thyroxine (100 μg/100 g) at 48-hr intervals; all other rats were sham-injected. 1,1-DCE was given po in mineral oil 24 hr after the last T_4 dose; controls received only mineral oil. Animals were killed at 2, 4, and 8 hr. Liver GSH contents were lowered about 55% by both fasting and T_4 while GSH transferase activities were lowered about 20% by fasting and 35% by T_4. Only T_4 pretreatment lowered alcohol dehydrogenase activities. Liver injury (i.e., serum glutamate pyruvate transaminase, histology) after 1,1-DCE was minimal in fed rats, moderate in fasted rats, and intermediate in T_4 rats. Fasted rats showed a more pronounced depletion of liver GSH after 1,1-DCE than T_4 rats and only in fasted rats did the toxicant decrease activities of the detoxification enzymes. Hypoglycemia after 1,1-DCE occurred in fed rats, but more rapidly in T_4 rats. In contrast, fasted rats unexpectedly became hyperglycemic after the toxicant. Patterns of body temperature change after the toxicant, which might be due to its metabolites, were dissimilar. Hypothermia was not observed in fed rats, was only transiently evident in T_4 rats, but occurred rapidly within 1 hr in fasted rats and steadily became more severe. The dissimilar patterns of liver enzyme and body temperature and serum glucose change after the toxicant in the three groups are indicative of different pathways of injury potentiation by fasting and hyperthyroidism. © 1988 Academic Press, Inc.

1,1-Dichloroethylene (1,1-DCE) is an interesting model hepatotoxicant because multiple different conditions or pretreatments which are known to affect cell constituents involved in the metabolism of 1,1-DCE (Fig. 1) modulate its acute hepatotoxicity (Reynolds *et al.*, 1975; Szabo *et al.*, 1977; Anderson *et al.*, 1980; Masuda and Nakayama, 1983). These pretreatments provide a way to probe the cause–effect sequence of events leading to cell injury. 1,1-DCE is used for the synthesis of plastic film and has been identified in industrial sewage treatment effluents, well water, and treated drinking water (Coleman *et al.*, 1976).

Fasting and excess thyroxine are two pretreatments which markedly enhance the hepatotoxicity of 1,1-DCE (Jaeger *et al.*, 1974; Szabo *et al.*, 1977; Jaeger *et al.*, 1977). Both of these pretreatments lower liver contents of GSH (Jaeger *et al.*, 1977) which, as illustrated in Fig. 1, is needed for the formation of conjugates with reactive metabolites of 1,1-DCE (Liebler *et al.*, 1985). However, the mecha-

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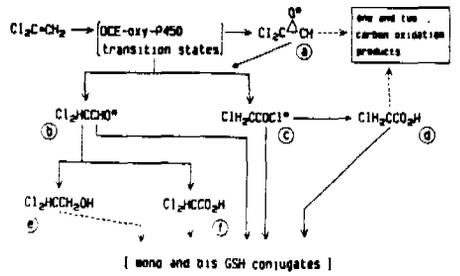

FIG. 1. Pathways for metabolism of 1,1-DCE by a cytochrome *P*-450-dependent Phase I reaction to reactive intermediates (a, b and c) that can be further converted by hydrolysis or phase II oxidation, reduction (to intermediates d, e and f) and finally conjugation with one or two molecules of GSH. Alcohol and aldehyde dehydrogenases presumably convert the dichloroacetaldehyde intermediate (b) to alcohol (e) and acid (f) intermediates. Glutathione transferases may facilitate the conjugation with GSH. Intermediates capable of binding to cell constituents are marked with an *. Modified from Costa and Ivanetich (1984).

nisms underlying the injury enhancement are poorly understood. In the two studies of thyroxine enhancement (Szabo *et al.*, 1977; Jaeger *et al.*, 1977), 1,1-DCE was administered at a very toxic dose (2000 ppm for 4 hr) that was lethal to 60% of the fasted rats within 3 days, whereas 75% of the hyperthyroid, fasted rats died—most during the 4-hr 1,1-DCE exposure period.

This study was designed to compare the responses of fed, fasted, and thyroxine (T_4)-pretreated rats to 50 mg 1,1-DCE/kg which produces moderate liver injury in fasted rats and is not lethal (Moslen and Reynolds, 1985). Body temperature changes were monitored because 1,1-DCE was reported to produce a rapid and dose-dependent hypothermia in mice (Masuda and Nakayama, 1983). Patterns of GSH depletion in liver and other tissues were examined because GSH conjugates are the major metabolites of 1,1-DCE (Liebler *et al.*, 1985), and because covalent binding of 1,1-[^{14}C]DCE in mice has been reported highest in kidney, liver, and lung with smaller amounts in heart and gut (Okine *et al.*, 1985). Effects of the pretreatments and 1,1-DCE on cytochrome *P*-450 content were not evaluated in this *in vivo* study. Isozymes of cytochrome *P*-450 differ widely in their rates of oxidizing 1,1-DCE, particularly with regard to formation of the very reactive dichloroacetaldehyde (metabolite b in Fig. 1) (Liebler and Guengerich, 1983). Therefore, an in depth *in vitro* study would be required to determine the effects of these pretreatments and 1,1-DCE on oxidation of 1,1-DCE to reactive metabolites. However, we did investigate effects of the pretreatments and 1,1-DCE on other liver constituents (including glutathione transferases, alcohol and aldehyde dehydrogenases) potentially involved in the detoxification of reactive intermediates of 1,1-DCE. Liver injury was monitored by measuring serum activities of liver-derived enzymes and bilirubin content.

MATERIALS AND METHODS

Chemicals. 1,1-DCE of 99% purity was obtained from Aldrich Chemical Co. (Milwaukee, WI). Thyroxine, NAD, glutathione, and propionaldehyde were obtained from Sigma Chemical Co. (St. Louis, MO). The glutathione transferase substrates, chloro-2,4-dinitrobenzene (CDNB), 3,4-dichloronitrobenzene (DCNB), and 1,2-epoxy 3-(*p*-nitrophenoxy)-propane (ENPP), were obtained from Aldrich or Sigma. CDNB and DCNB were recrystallized in ethanol-water twice before use.

Animals. Male Sprague–Dawley rats (Timco, Indianapolis, IN) weighing 230–300 g were used. The rats were housed in wire-floored cages suspended over absorbent paper in a 12-hr light/dark cycle animal room for at least 1 week prior to beginning treatments. Purina Lab Chow was provided *ad libitum*.

Experimental protocol. Animals were randomly assigned to the fed group, the fasted group, or the thyroxine group. Animals in the thyroxine group received three sc injections of thyroxine (100 μg/100 g) dissolved in alkaline saline at 48-hr intervals with the last injection 24 hr prior to the experiment. Animals in both the fed and fasted groups received three injections of alkaline saline. Measurements of serum T_4 levels by radioimmunoassay (Mallinckrodt, SPAC T_4 RIA kit, Mallinckrodt, Inc., St. Louis, MO) verified that this T_4 pretreatment regimen produced a fourfold elevation of serum T_4 level (i.e., 16.4 μg T_4/dl compared to 4.0 μg T_4/dl in fed controls). Animals in the fasted group had their food removed at 4 pm on the day prior to the experiment.

At 8 AM on the morning of the experiment, all animals were weighed and randomly assigned to 1,1-DCE treat-

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ment or control groups, and food was removed from the ed and T_4 groups. Between 9 AM and 11 AM, 1,1-DCE as administered at a dose of 50 mg/kg by gavage in 2 ml nineral oil/kg. Treatment controls received mineral oil.

Body temperatures were measured with an electronic ectal thermometer (Harvard Bioscience, South Natick, IA) at 30-min intervals for the first 2 hr and at 60-min ntervals for the next 6 hr. Fasted and T_4 animals were illed at 2, 4, or 8 hr after treatment with 1,1-DCE. Fed its were killed only at 4 and 8 hr, since other studies ave indicated little difference between 2 and 4 hr in the iochemical parameters of interest such as serum glu-ose, glutathione, and the cytoplasmic enzyme levels Moslen *et al.*, 1987). Control animals were killed at the eginning and end of the experimental period to provide early" control values and "late" control values for the - and 4-hr experimental animals and the 8-hr experimental animals, respectively. Diurnal variation of liver lutathione is less than 5% in fed animals and less than 5% in fasted animals between 9 AM and 1 PM (Jaeger *et l.*, 1973; Reynolds *et al*, 1980). Animals were anesthe-ized with ether.

Liver, kidney, and testis were homogenized in 5 vol nd heart, lung, and small intestine in 10 vol of buffered) 25 M sucrose, 0.01 mM KPO_4 (pH 7.4), and cytosol was prepared by differential centrifugation as previously de-scribed (Moslen and Reynolds, 1985). Liver cytosol con-aining 0.33 mM dithiothreitol was stored at −80°C for iochemical assays. Preliminary studies indicated that dithiothreitol addition provided excellent preservation of enzyme activities and had no effect on toxicant-induced alterations.

Biochemical analyses. Serum glucose and bilirubin concentrations and glutamate pyruvate transaminase (GPT) activities were measured with Sigma reagent kits Nos. 16, 605, and 57, respectively. Protein was measured by the Lowry *et al.* (1951) method with bovine serum albumin as the standard. Reduced glutathione content was estimated with Ellman's reagent as previously described (Moslen and Reynolds, 1985). Glutathione transferase activities towards CDNB and DCNB were measured spectrophotometrically at 25°C, and ENPP at 30°C, according to Habig *et al.* (1974) and Younes *et al.* (1980a), respectively. Alcohol dehydrogenase was assayed in the direction ethanol → acetaldehyde according to Crow *et al.* (1977). Aldehyde dehydrogenase was assayed according to Lindahl (1979) using propionaldehyde as substrate and NAD as coenzyme.

Statistical analysis. The data obtained were analyzed by one-way analysis of variance using a statistical package (STATSOFT) for personal computers; when differences were found at $p < 0.05$, individual comparisons between a treatment group and its respective "early" or "late" control were evaluated by the *t* test.

RESULTS

As illustrated in Fig. 2, body temperatures of fed rats were not altered by 1,1-DCE.

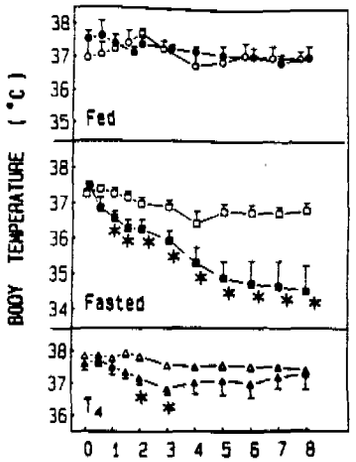

FIG. 2. Time course of body temperature changes in fed, fasted, and thyroxine (T_4)-pretreated rats given 50 mg 1,1-DCE in mineral oil (filled symbols) or mineral oil (open symbols). Values are means ± SE of four rats per group. Where error bars are not shown, the SE lies within the symbol. Asterisks near symbols of 1,1-DCE-treated rats indicate values that were significantly lower than their respective controls at $p < 0.05$.

Fasted rats showed a decline in body temperature within 1 hr after 1,1-DCE administration; the hypothermia was persistent and progressive. In contrast, 1,1-DCE had a slight and transient hypothermic effect in T_4 rats that was statistically significant only at 2 and 3 hr.

Figures 3 and 4 compare the relative magnitudes of liver injury in the animals given 1,1-DCE as quantitated by the elevation in SGPT activities and serum bilirubin contents. The change in SGPT was slight in the fed group, most severe in the fasted group, and intermediate in the T_4 group. Only the fasted group showed statistically significant increases in bilirubin.

Histological changes in the centrilobular regions of livers of fasted and T_4 rats after 1,1-DCE are shown in Fig. 5. The pretreatments of fasting and T_4 administration did not alter liver histology in control animals (i.e., see Fig. 5A). At 8 hr after 1,1-DCE, morphological changes were confined to an area of three to

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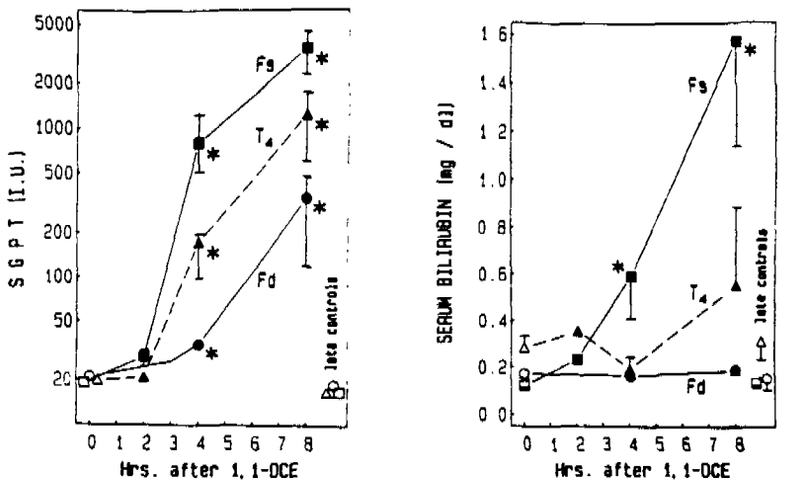

FIGS. 3 and 4. Time course of changes in serum GPT activities and bilirubin contents of fed (Fd), fasted (Fs), and thyroxine (T_4)-pretreated rats given 50 mg 1,1-DCE/kg in mineral oil (filled symbols) or mineral oil (open symbols). Values are means ± SE of four rats per group. Where error bars are not shown, the SE lies within the symbols. Asterisks near symbols of 1,1-DCE-treated rats indicate values that were significantly higher ($p < 0.05$) than their early pretreatment control at 2 and 4 hr or late pretreatment control at 8 hr.

five cell layers surrounding the central vein in livers of T_4 pretreated animals (Fig. 5B) while livers of fasted rats showed extensive centrilobular necrosis (Fig. 5C). Morphological changes were not consistently observed in liver sections from fed rats 8 hr after 1,1-DCE (Fig. 5D), even though serum GPT activities were increased by approximately 19-fold.

Patterns of serum glucose change were markedly different in the fed and T_4 animals compared to the fasted animals, as indicated in Fig. 6. Fed rats showed no appreciable change by 4 hr after 1,1-DCE and then serum glucose decreased to 81% of the fed late controls by 8 hr. T_4 rats showed a much more abrupt decrease in serum glucose values after toxicant administration; values at 2 hr were 66% of the T_4 early control values. In contrast, the fasted rats became hyperglycemic after 1,1-DCE; values at 4 hr were 150% of fasted controls.

Figure 7 compares the different patterns of change in liver GSH. Note that the livers of both the fasted and T_4 control rats had less than 60% as much GSH per kilogram body weight as did the livers of the fed controls. The lesser amounts of GSH in the livers of the fasted and T_4 controls reflect, in part, their smaller average liver weights of 30.2 and 34.0 g/kg body wt, respectively (compared to 38.9 for the fed control). However, their smaller livers did not fully account for the

FIG. 5. Livers of rats given mineral oil (A) or 50 mg 1,1-DCE/kg (B, C, D) 8 hr previously. (c, central vein; p, portal). H&E, ×325. (A) Pretreatment by T_4 administration (or fasting, not shown) did not produce morphological alterations in liver parenchyma. (B) Pale, enlarged vacuolated cells occur up to five cell layers away from the central vein (arrow) in T_4-pretreated animals. (C) Extensive liver necrosis and congestion surround the central vein and extend toward the midzonal region in fasted animals. (D) Appreciable alterations to centrilobular liver parenchyma were not observed in fed animals.

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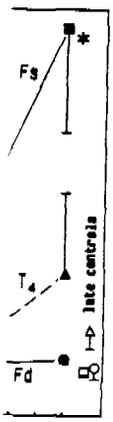

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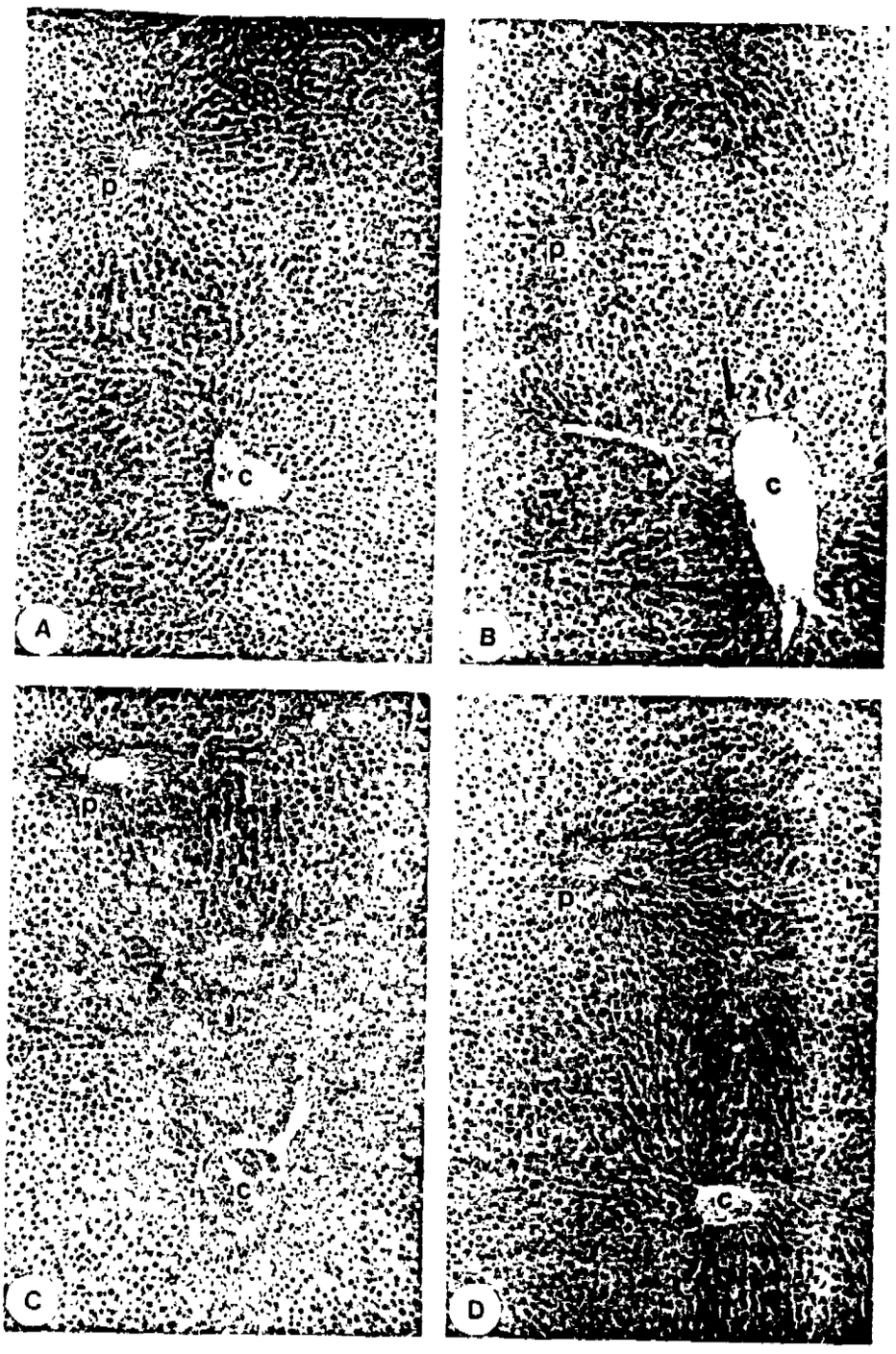

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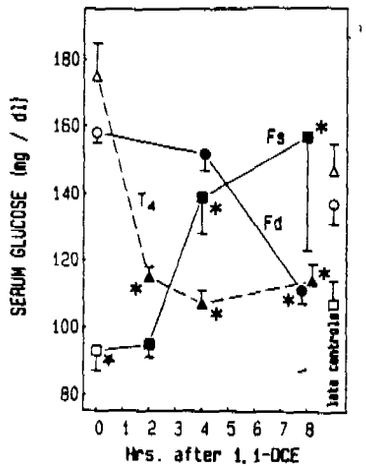

FIG. 6. Time course of changes in blood glucose contents of fed (Fd), fasted (Fs), and thyroxine (T_4)-pretreated rats given 50 mg 1,1-DCE/kg in mineral oil (filled symbols) or mineral oil (open symbols). Values are means ± SE of four rats per group. Star near the symbol for the fasted early control value indicates difference at $p < 0.05$ from the fed early control value. Asterisks near symbols of 1,1-DCE-treated rats indicates values that were significantly different ($p < 0.05$) from their early pretreatment control at 2 and 4 hr or late pretreatment control at 8 hr.

difference in hepatic GSH, since when hepatic GSH content is evaluated in terms of micromoles GSH/gram liver, values of liver GSH in the fasted and T_4 rats were still about 70% that of fed rats.

1,1-DCE caused an early depletion of hepatic GSH to about 50–60% control values in both the fed and T_4 groups and a relatively greater magnitude of decrease to about 30% control values in fasted rats. The patterns of hepatic GSH change at 8 hr after 1,1-DCE were quite different, with a small but significant rebound above late control values in the fed group compared to sharper rebounds above the nadir in the fasted and T_4 groups. Another difference in the response of the three groups to the toxicant was that average amounts of hepatic GSH depletion at the early time point in the fed and fasted groups were similar with decreases of 107 and 97 mmol/kg, respectively, while the amount of average early decrease in the T_4 group was less at 67 mmol/kg.

Kidney and lung patterns of GSH change are shown in Fig. 8. Fasting did not alter kidney or lung GSH content, unlike the marked effect of fasting on liver content of this nucleophile. T_4 administration led to a statistically significant increase in kidney GSH content, but a decrease in lung GSH content compared to fed controls. 1,1-DCE had no evident effect on kidney GSH in fed rats. However, the toxicant had opposite effects on kidney GSH in the other two groups; these were an increase to 123% of control values by 8 hr in the fasted rats but a decrease to 82% by 4 hr in T_4 rats. Lung GSH contents were diminished after 1,1-DCE in fed and fasted rats, but were not appreciable altered in T_4 rats.

Patterns of GSH change in testis and heart and in small intestine are presented in Figs. 9 and 10, respectively. Fasting produced significant increases in GSH in both testis and heart compared to fed controls while T_4 pretreatment increased GSH content only in the heart. In contrast to the effects of 1,1-DCE on liver, kidney, and lung GSH levels, testis and heart GSH were not appreciably altered by 1,1-DCE. Small intestine GSH contents were similar in the pretreated and fed controls with 1,1-DCE administration producing a GSH decrease only in the fasted group, to 76% of fasted control value by 4 hr with a subsequent rebound by 8 hr.

Tables 1 and 2 detail the changes measured in liver cytosolic enzymes that potentially have a role in the detoxification of reactive epoxide, alcohol, aldehyde, or acid metabolites of 1,1-DCE. Glutathione transferase activities toward the three substrates were lower in the fasted controls than the fed controls, and even lower in the T_4 controls. 1,1-DCE administration to fed rats led to a small decrease in this enzyme activity toward one substrate (ENPP), while marked decreases were consistently found after toxicant administration toward all substrates in fasted rats.

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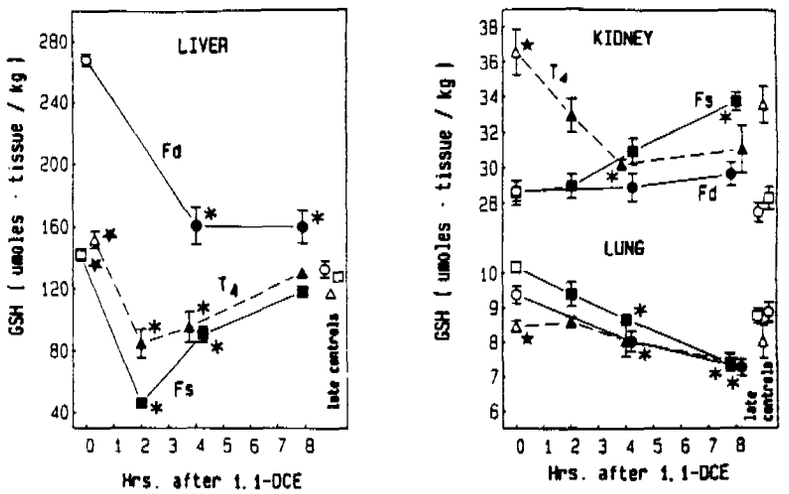

FIGS. 7 and 8. Time course of changes in liver, and kidney and lung GSH of fed (Fd), fasted (Fs), and thyroxine (T_4)-pretreated rats given 50 mg 1,1-DCE kg in mineral oil (filled symbols) or mineral oil (open symbols). Values are means ± SE of four rats per group. Where error bars are not shown, the SE lies within the symbol. Stars near symbols of early control values indicate values that were different at $p < 0.05$ from the fed early controls. Astensks near symbols of 1,1-DCE-treated rats indicate values that were significantly different ($p < 0.05$) from their early pretreatment control at 2 and 4 hr or late pretreatment control at 8 hr.

In contrast, 1,1-DCE had no appreciable effect on glutathione transferases of T_4 rats. The most striking change observed in the alcohol and aldehyde dehydrogenase activity was that the T_4 control animals had just 39% as much hepatic alcohol dehydrogenase activities as the fed controls. 1,1-DCE administration consistently led to decreases in both dehydrogenase activities in the fasted rats, but had no evident effect in either the fed or T_4 rats.

DISCUSSION

This study demonstrated that pretreatment of rats with sufficient T_4 to produce hyperthyroidism enhanced the hepatotoxicity of a nonlethal dose of 1,1-DCE. The enhancing effect of T_4 pretreatment on 1,1-DCE hepatotoxicity was less than that of fasting pretreatment.

We examined the effects of fasting and T_4 pretreatment on several liver constituents whose alteration could affect the detoxification of 1,1-DCE reactive metabolites. Liver GSH contents were lowered to a similar extent by fasting and T_4. Fasting slightly decreased aldehyde dehydrogenase activities, and in accord with the study of Mezey and Potter (1981), T_4 diminished alcohol dehydrogenase activities more than 50%. In addition, we found for the first time that T_4 produced a substantial decrease in cytosolic glutathione transferase activities. The T_4 effect on the glutathione transferases was evident with the universal substrate, CDNB, and also with the substrates, DCNB and ENPP, reported to be more specific for certain isozymes of the enzyme (Habig *et al.*, 1974). A prior study in Wistar rats had reported an increase in epoxide transferase activity and no change in aryl transferase activity following a 3-day triiodothyronine pretreatment (Younes *et al.*, 1980b).

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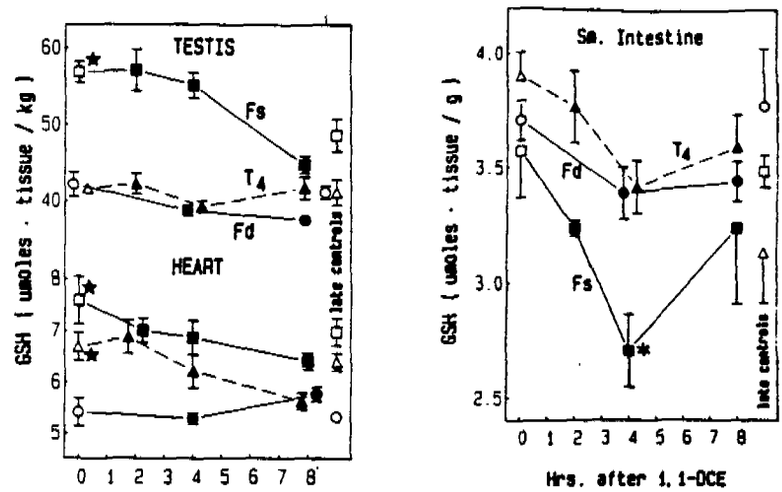

FIGS. 9 and 10. Time course of changes in testis and heart, and small intestine GSH of fed (Fd), fasted (Fs), and thyroxine (T_4)-pretreated rats given 50 mg 1,1-DCE/kg in mineral oil (filled symbols) or mineral oil (open symbols). Values are means ± SE of four rats per group. Where error bars are not shown, the SE lies within the symbol. Stars near symbols of early control values indicate values that were different at $p < 0.05$ from the fed early controls. Asterisks near symbols of 1,1-DCE-treated rats indicate values that were significantly different ($p < 0.05$) from their early pretreatment control at 2 and 4 hr or late pretreatment control at 8 hr.

Our observation of more severe 1,1-DCE-induced liver injury in the fasted than the T_4-pretreated rats, despite their similar GSH content and the lesser effect of fasting on the detoxification enzymes (glutathione transferase and alcohol dehydrogenase), suggests that fasting potentiates the hepatotoxicity of 1,1-DCE by different pathways than T_4. This difference in injury potentiation might be due to differences in 1,1-DCE metabolism, i.e., in the rate or extent of 1,1-DCE activation to reactive intermediates or the subsequent detoxification of these reactive species.

Several findings of our study are consistent with possible differences in 1,1-DCE metabolism between the fasted and T_4 groups. First, 1,1-DCE had a more pronounced hypothermic effect in fasted rats than in T_4 rats. Masuda and Nakayama (1983) proposed that the dose-dependent hypothermic effect of 1,1-DCE was due to its metabolites because they found that administration of cytochrome *P*-450 inhibitors shortly before 1,1-DCE poisoning prevented hypothermia. In addition, they found that two of 1,1-DCE's oxidized metabolites, chloroacetyl chloride and monochloroacetic acid (c and d in Fig. 1), rapidly caused hypothermia. Second, 1,1-DCE caused a more pronounced depletion of hepatic GSH in fasted than T_4 rats which suggests that metabolite conjugation with GSH occurs at a different rate or extent in fasted than T_4 rats.

Third, only in the fasted rats did 1,1-DCE cause a decrease in liver glutathione transferase activities. Moslen and Reynolds (1985) proposed that the decrease in hepatic glutathione transferases of fasted animals given 1,1-DCE could be due to the "scavenger" function of the enzyme. Kinetics studies of glutathione transferase isozymes indicate that, under normal physiologic conditions, GSH binds to the enzyme before the electrophilic substrates (Pabst *et al.*, 1974). Low GSH concentrations facilitate its scavenging function *in vitro*, possibly by allowing the re-

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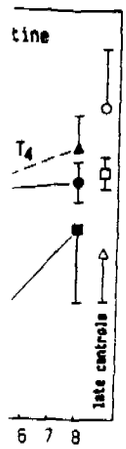

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TABLE 1

EFFECT OF FASTING, THYROXINE AND 1,1-DCE ON LIVER CYTOSOLIC GLUTATHIONE TRANSFERASE ACTIVITIES

			Glutathione transferase		
Pretreatment	Treatment	Time (hr)	CDNB	DCNB (μmol·liver/kg·min)	ENPP
Fd	oil	2	3307 ± 120	143 ± 11	65.6 ± 2.0
Fd	1,1-DCE	4	2990 ± 226 (91%)[a]	135 ± 9 (94%)[a]	53.8 ± 0.9 (82%)[a]++
Fs	oil	2	2654 ± 108 (80%)[a,*]	115 ± 4 (80%)[a,*]	48.5 ± 2.8 (74%)[a,**]
Fs	1,1-DCE	2	1836 ± 99 (69%)[b]††	67 ± 3 (58%)[b]††	30.1 ± 1.6 (62%)[b]++
Fs	1,1-DCE	4	1793 ± 86 (68%)[b]††	70 ± 4 (61%)[b]††	30.6 ± 0.8 (63%)[b]++
Fd T_4	oil	2	2147 ± 19 (65%)[a,**]	82 ± 1 (57%)[a,**]	43.3 ± 1.3 (66%)[a,**]
Fd T_4	1,1-DCE	2	1984 ± 76 (92%)[c]	77 ± 5 (94%)[c]	40.0 ± 3.1 (92%)[c]
Fd T_4	1,1-DCE	4	2091 ± 80 (97%)[c]	80 ± 2 (97%)[c]	39.6 ± 5.4 (91%)[c]

Note. Values are means ± SE of four animals per group.
[a] Percentage of Fd-oil control group.
[b] Percentage of Fs-oil control group.
[c] Percentage of T_4-oil control group.
* $p < 0.05$ compared to Fd control group.
** $p < 0.01$ compared to Fd control group.
† $p < 0.05$ compared to pretreatment control group.
†† $p < 0.01$ compared to pretreatment control group.

TABLE 2

EFFECT OF FASTING, THYROXINE AND 1,1-DCE ON LIVER CYTOSOLIC ENZYME ACTIVITIES

			Alcohol dehydrogenase	Aldehyde dehydrogenase
Pretreatment	Treatment	Time (hr)	Ethanol (μmol·liver/kg·min)	Propionitrile (μmol·liver/kg·min)
Fd	oil	2	45.5 ± 3.2	32.1 ± 1.3
Fd	1,1-DCE	4	44.7 ± 2.2 (98%)[a]	32.6 ± 1.7 (102%)[a]
Fs	oil	2	39.6 ± 3.5 (87%)[a]	25.4 ± 2.4 (79%)[a,*]
Fs	1,1-DCE	2	32.1 ± 3.5 (81%)[b]†	21.6 ± 0.1 (85%)[b]†
Fs	1,1-DCE	4	32.1 ± 1.9 (81%)[b]†	18.8 ± 2.1 (74%)[b]††
Fd T_4	oil	2	17.7 ± 0.9 (39%)[a,**]	30.7 ± 1.0 (96%)[a]
Fd T_4	1,1-DCE	2	16.4 ± 1.0 (93%)[c]	33.1 ± 2.9 (108%)[c]
Fd T_4	1,1-DCE	4	20.5 ± 3.1 (116%)[c]	30.6 ± 2.6 (100%)[c]

Note. Values are means ± SE of four animals per group.
[a] Percentage of Fd-oil control group.
[b] Percentage of Fs-oil control group.
[c] Percentage of T_4-oil control group.
* $p < 0.05$ compared to Fd control group.
** $p < 0.01$ compared to Fd control group.
† $p < 0.05$ compared to pretreatment control group.
†† $p < 0.01$ compared to pretreatment control group.

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active electrophilic substrate to bind first to enzyme not "protected" by a bound GSH (Wolkoff *et al.*, 1979). The lesser magnitude of GSH depletion in the T_4 compared to the fasted rats after 1,1-DCE may account for the absence of 1,1-DCE-induced decrease of glutathione transferase activities in the T_4 rats.

Blood glucose was not monitored in prior studies of 1,1-DCE toxicity. We expected a hypoglycemic effect in all groups because of the need to use hepatic glycogen stores for metabolism of 1,1-DCE—specifically, to provide reducing equivalents for the regeneration of the NADPH required for the first oxidation of 1,1-DCE by a NADPH–cytochrome *P*-450 reaction (Costa and Ivanetich, 1984). Since hyperthyroidism is associated with low hepatic glycogen stores, it is logical that hypoglycemia occurred more rapidly after 1,1-DCE in the T_4 group than the fed group. Unexpected was the hyperglycemia in the fasted group after 1,1-DCE. It is possible that 1,1-DCE damages liver enzymes which regulate hepatic gluconeogenesis or the retention of glucose by the hepatocyte. Alternatively, 1,1-DCE could cause the hyperglycemia by an injurious effect on the pancreatic β cell as has been observed by Hinson *et al.* (1983, 1984) in mice poisoned with acetaminophen. However, we have not observed appreciable light microscopic alterations in the pancreas of 1,1-DCE-treated fasted rats (Kanz and Moslen, unpublished observation).

In summary, the lack of concordance in the responses of the fasted and T_4 rats to a moderately injurious dose of 1,1-DCE—specifically, the dissimilarities in the magnitude of GSH depletion, and in the onset and duration of hypothermia, as well as the opposite changes in serum glucose—indicates that these two pretreatments enhance 1,1-DCE hepatotoxicity by different mechanisms.

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