TOXICOLOGY AND APPLIED PHARMACOLOGY 62, 1-10 (1982)

The Effect of Repeated Vinyl Chloride Exposure on Rat Hepatic Metabolizing Enzymes[1]

JULIE T. DU,[2] MICHAEL T. TSENG, AND CARLO H. TAMBURRO[3]

Liver Research Center, Division of Digestive Diseases and Nutrition, Departments of Medicine and Anatomy, and Regional Cancer Center, University of Louisville School of Medicine, Louisville, Kentucky 40292

Received July 17, 1980; accepted September 12, 1981

The Effect of Repeated Vinyl Chloride Exposure on Rat Hepatic Metabolizing Enzymes. DU, J. T., TSENG, M. T., AND TAMBURRO, C. H. (1982). *Toxicol. Appl. Pharmacol.* **62**, 1-10. Sprague-Dawley rats were exposed to 2.8% vinyl chloride for 2 (70 hr), 4 (140 hr), and 6 (210 hr) weeks to determine the sequential biochemical changes related to the oxidation and detoxification ability of hepatic tissue. Glutathione-*S*-transferase(s) activity using 1,2-epoxy-(*p*-nitrophenoxy)propane and *p*-nitrobenzyl chloride as substrates was elevated 17 to 24, 28, and 35 to 42% after 2, 4, and 6 weeks of exposure, respectively, suggesting enzyme(s) induction. Reduced glutathione, the major substrate required to conjugate the toxic metabolites of vinyl chloride, was also consistently elevated. Similarly, the activity of glutathione reductase, the enzyme necessary for the regeneration of reduced glutathione from its oxidized form, was also increased following vinyl chloride exposure. Cytochromes *P*-450, the major protein involved with vinyl chloride metabolism, was reduced after vinyl chloride exposure, confirming reports of others that vinyl chloride metabolites destroy *P*-450. No abnormalities of standard clinical biochemical blood tests of liver function were found during 6 weeks of vinyl chloride exposure. The only consistent ultrastructural modification was the dilation of endoplasmic reticulum. The biochemical and ultrastructural alterations could reflect early hepatocellular adaptation to vinyl chloride exposure.

Vinyl chloride, at high concentrations, has been shown to be carcinogenic in both laboratory animals (Maltoni and Lefemine, 1975; Viola *et al.*, 1971) and man (Creech and Johnson, 1974). Present data support the metabolism of vinyl chloride by hepatic microsomal mixed-function oxidase system into toxic intermediates, chloroethylene oxide (Bolt *et al.*, 1975; Hefner *et al.*, 1975; Kappus *et al.*, 1976) and chloroacetaldehyde (Gross and Freiberg, 1969). These two intermediates are considered to be the ultimate carcinogens (Barbin *et al.*, 1975; Jaeger *et al.*, 1974b; Van Duuren, 1975), to be mutagenic in bacterial systems (Elmore *et al.*, 1976; Greim *et al.*, 1975; Malaveille *et al.*, 1975; McCann *et al.*, 1975), to act as an alkylating agent by reacting with adenosine (Barbin *et al.*, 1975) and cytidine (Laib and Bolt, 1978), and to bind with protein (Bolt *et al.*, 1976; Kappus *et al.*, 1976; Watanabe *et al.*, 1978). Detoxification of these metabolites occurs mainly by conjugation with glutathione and is catalyzed by hepatic glutathione transferases; the conjugates are excreted in the urine as substituted cysteine derivatives (Watanabe *et al.*, 1976b,c; Green and Hathway, 1975, 1977). Chloroacetaldehyde can be further oxidized to chloro-

[1] This work was supported by a grant from the Manufacturing Chemists Association, Washington, D.C. Portions of this study have been presented (Fed. Proc. **37**, 1545, 1978).

[2] Present address: Clement Associates, Inc., 1010 Wisconsin Avenue, N.W., Suite 660, Washington, D.C. 20007.

[3] Address requests for reprints to: Carlo H. Tamburro, University of Louisville.

0041-008X/82/010001-10$02.00/0

acetic acid (Hefner *et al.*, 1975). These data are compiled in a metabolic scheme in Fig. 1 as an updated hypothesized metabolic fate of vinyl chloride in the adult rat. The metabolism of chloroethylene oxide via epoxide hydratase is not listed in the scheme because its product has not been identified.

There have been a few *in vivo* studies concerning the effects of vinyl chloride exposure on hepatic concentrations of glutathione (Hefner *et al.*, 1975; Watanabe *et al.*, 1976c; Du and Tamburro, 1978), cytochromes *P*-450 (Reynolds *et al.*, 1975) and on mixed-function oxidase activity (Drew *et al.*, 1975; Reynolds *et al.*, 1975). However, information about the sequential alterations in hepatic oxidation and detoxification of vinyl chloride, during prolonged exposure, simulating the occurrence in workers, is still lacking. It was reported previously that the enzymatic changes in rat liver following prolonged exposure to vinyl chloride were similar to those found in rat hepatoma (Du and Tamburro, 1976; Du *et al.*, 1979). The sequential biochemical changes related to the hepatic oxidation and detoxification of vinyl chloride following prolonged exposure are reported here.

METHODS

Animals and experimental design. Eight- to ten-week-old Sprague–Dawley male rats (~300 g), supplied by Laboratory Supply of Indianapolis, Indiana, were randomized prior to the experiment into three groups: a vinyl chloride-exposed group and the air-exposed group housed in identical chambers and a second control group housed in the University's Central Animal Care Center. The exposure level was 28,000 ppm vinyl chloride, 7 hr/day, 5 days/week for 2, 4, and 6 weeks. The exposure chambers were 4400-liter airtight vats. Vinyl chloride (~300 to 340 g) was added to the vat to give a time-weighted average concentration of 28,000 ± 1000 ppm. The chamber air was changed daily and the vinyl chloride concentration was determined by gas chromatography. The air was constantly circulated by a stirrer. The rats' respirations had negligible effect on the composition of the chamber's atmosphere because of the chamber's large volume. Animals were fed on standard laboratory chow pellets *ad libitum*.

All animals were anesthetized with ether, blood was drawn from the inferior vena cava, and the animals were killed approximately each day.

NADPH, glutathi obtained from Sigm Missouri; 1,2-epox purchased from Eas New York; *p*-nitrob Matheson, Coleman Jersey; benzphetam Company, Kalamaz was used throughou

Sample preparati Homogenates and s as described previou was prepared from a preparation. The re liquid nitrogen and 450 concentrations crosomal fractions t tathione (GSH) con *S*-transferase, glutat tion oxidase activiti fractionated frozen tissue, the livers from frozen in an identic time. Cytochromes Sato, 1964), nonprot Lindsay, 1968), ar (Carlberg and Man the microsomal or scribed previously (

Glutathione-*S*-tra using the 100,000 × 3-(*p*-nitrophenoxy)p were the substrates ase and glutathione- spectively. Enzyme a by others (Habig *et* All assays were linea and timed for at lea (*p*-nitrophenoxy)pro were prepared in abs centration in the inc function oxidase act somal fraction by n in the NADPH-dep benzphetamine (Lu was determined by t The serum clinical notransferase, alanir phatase, bilirubin, determined by Tech computer (SMAC)

Light and electro were removed under cubes, placed imme troxide (pH 7.4), a

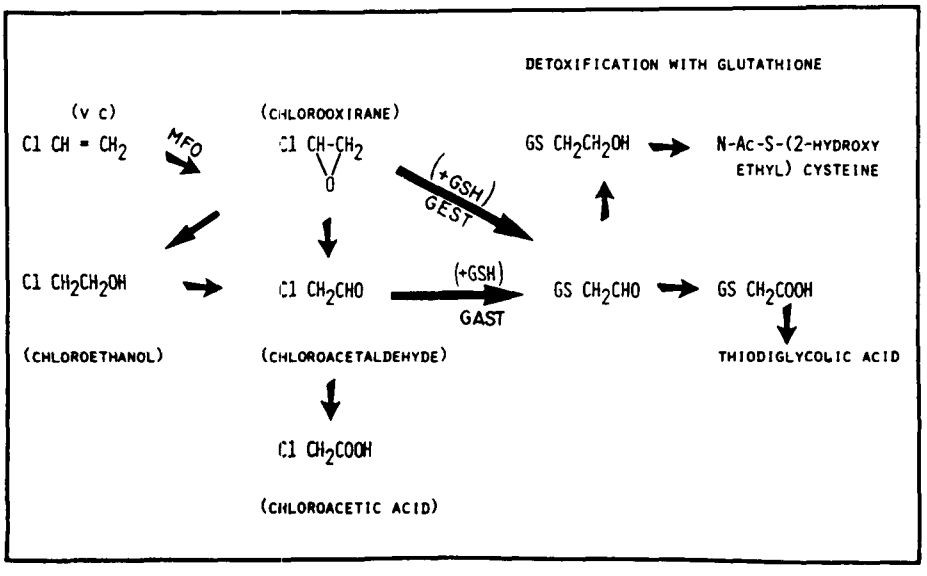


FIG. 1. The proposed metabolic fate of vinyl chloride. (GSH, glutathione; MFO, mixed-function oxidase; VC, vinyl chloride; GEST, glutathione *S*-epoxide transferase; and GAST, glutathione *S*-aldehyde transferase).

killed approximately 20 hr after exposure at 1:00 pm each day.

NADPH, glutathione, and glutathione disulfide were obtained from Sigma Chemical Company, St. Louis, Missouri; 1,2-epoxy-3-(*p*-nitrophenoxy)propane was purchased from Eastman Kodak Company, Rochester, New York; *p*-nitrobenzyl chloride was obtained from Matheson, Coleman and Bell, East Rutherford, New Jersey; benzphetamine was donated by the UpJohn Company, Kalamazoo, Michigan. Double-distilled water was used throughout.

Sample preparation and biochemical determination. Homogenates and subcellular fractions were prepared as described previously (Du *et al.*, 1979). Each sample was prepared from a single organ and kept at 4°C during preparation. The remaining liver was frozen rapidly in liquid nitrogen and stored at −70°C. Cytochromes *P*-450 concentrations were determined in the frozen microsomal fractions the day following sacrifice. The glutathione (GSH) concentration, as well as glutathione-*S*-transferase, glutathione reductase, and mixed-function oxidase activities were determined in the freshly fractionated frozen liver. For the assays using frozen tissue, the livers from control and experimental rats were frozen in an identical manner for the same length of time. Cytochromes *P*-450 concentration (Omura and Sato, 1964), nonprotein sulfhydryl content (Sedlak and Lindsay, 1968), and glutathione reductase activity (Carlberg and Mannervik, 1975) were determined in the microsomal or cytosol fractions by methods described previously (Du *et al.*, 1979).

Glutathione-*S*-transferase activity was determined using the 100,000 × *g* supernatant fraction. 1,2-Epoxy-3-(*p*-nitrophenoxy)propane and *p*-nitrobenzyl chloride were the substrates for glutathione-*S*-epoxide transferase and glutathione-*S*-aralkyl transferase (GAST), respectively. Enzyme activity was determined as described by others (Habig *et al.*, 1974; Kaplowitz *et al.*, 1975). All assays were linear functions of protein concentration and timed for at least 2 min. Solutions of 1,2-epoxy-3-(*p*-nitrophenoxy)propane and *p*-nitrobenzyl chloride were prepared in absolute ethanol; the final ethanol concentration in the incubation mixture was 0.5%. Mixed-function oxidase activity was estimated in the microsomal fraction by measuring NADPH disappearance in the NADPH-dependent demethylation reaction of benzphetamine (Lu *et al.*, 1972). The protein content was determined by the method of Lowry *et al.* (1951). The serum clinical liver tests including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, cholesterol, and triglyceride were determined by Technicon sequential multiple analyzer computer (SMAC) system.

Light and electron microscopy. Small strips of liver were removed under ether anesthesia, sliced into small cubes, placed immediately in ice-cold 1% osmium tetroxide (pH 7.4), and fixed for 2 hr at 4°C. Subsequently, samples were washed overnight in phosphate buffer, dehydrated in ascending alcohol, and embedded in Epon. Tissue blocks were polymerized at 60°C for 2 days. Thin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate before examination on a Philips 300 electron microscope. For ultrastructural analysis, three rats randomly selected from controls and groups exposed for 2, 4, and 6 weeks to vinyl chloride were studied.

For light microscopy, a block of tissue was fixed in buffered formalin and processed routinely for paraffin embedding. Sections 6 μm thick were stained with hematoxylin and eosin.

Statistical analysis. Analysis of variance was performed for the various groups at the different time periods and multiple comparisons were performed based on the results of the analysis of variance.

RESULTS

The protein content (mg protein/g liver) in the subcellular fractions in both control and vinyl chloride-exposed groups was the same throughout the exposure (data not shown); the enzymatic results, therefore, are expressed as micromoles of substrate converted per minute per milligram of protein.

There were no statistical differences between the normal and air-exposed groups in the glutathione and cytochromes *P*-450 contents or in any of the enzyme activities. The nonprotein sulfhydryl content (Table 1) was significantly elevated from 26 to 54% at 2, 4, and 6 weeks in the vinyl chloride-exposed group compared to both control groups. Although the nonprotein sulfhydryl content increased with exposure, the increases were not statistically significant. Glutathione reductase activity (Table 1) in the exposed group was increased by 53 to 77% at all three time periods. The increase in glutathione reductase was the same at 2 and 4 weeks of exposure but showed a further significant increase after 6 weeks of exposure. Glutathione-*S*-epoxide transferase (GEST, Table 1) and glutathione-*S*-aralkyl transferase (GAST, Table 1) activities were significantly higher than controls after 6 weeks of exposure, 37 and 45%, respectively. The cytochromes *P*-450 content, on the other hand,

TABLE I

SEQUENTIAL CHANGES IN HEPATIC NONPROTEIN SULFHYDRYL, CYTOCHROMES P-450 CONTENT AND ACTIVITIES OF GLUTATHIONE REDUCTASE AND GLUTATHIONE-*S*-TRANSFERASES (EPOXIDE AND ARALKYL) IN RATS EXPOSED TO VINYL CHLORIDE[a]

	Treatment	Time (weeks) 0	2	4	6
Nonprotein sulfhydryl (μmol/g liver)	Normal control	7.9 ± 0.3	7.8 ± 0.4[b]	7.1 ± 0.4[b]	6.9 ± 0.4[b]
	Vinyl chloride-exposed	—	9.4 ± 0.2[b,c]	10.2 ± 0.6[b,c]	11.4 ± 0.6[b,c]
	Air control	—	7.1 ± 0.3[c]	6.9 ± 0.4[c]	7.9 ± 0.3[c]
Glutathione reductase (100 × μmol/min/mg protein)	Normal control	5.0 ± 0.2	4.3 ± 0.4[b]	4.2 ± 0.4[b]	4.8 ± 0.3[b]
	Vinyl chloride-exposed	—	6.7 ± 0.6[b,c]	6.3 ± 0.5[b,c]	8.9 ± 0.7[b,c,d]
	Air control	—	4.5 ± 0.2[c]	3.7 ± 0.3[c]	5.3 ± 0.4[c]
GEST (100 × μmol/min/mg protein)	Normal control	9.1 ± 1.6	7.8 ± 0.4	7.5 ± 1.0	7.7 ± 1.0[b]
	Vinyl chloride-exposed	—	9.1 ± 1.7	9.7 ± 0.7[c]	11.0 ± 1.3[b,c]
	Air control	—	8.1 ± 1.2	6.3 ± 0.7[c]	8.4 ± 0.9[c]
GAST (10 × μmol/min/mg protein)	Normal control	2.4 ± 0.4	2.1 ± 0.3	1.9 ± 0.2	2.4 ± 0.3[b]
	Vinyl chloride-exposed	—	2.6 ± 0.4	2.4 ± 0.3	3.2 ± 0.1[b,c]
	Air control	—	2.2 ± 0.2	1.9 ± 0.2	2.1 ± 0.2[c]
Cytochrome *P*-450 (nmol/g liver)	Normal control	17.0 ± 3.7	17.1 ± 1.7	19.7 ± 1.3[b]	15.5 ± 1.4[b]
	Vinyl chloride-exposed	—	13.2 ± 1.1	15.3 ± 1.3[b]	10.6 ± 1.0[b,c]
	Air control	—	15.5 ± 1.3	19.6 ± 2.7	14.3 ± 1.8[c]

[a] Rats were exposed to 28,000 ppm of vinyl chloride; normal controls and the air controls were exposed to air only. Each number represents the mean and the SEM from a group of six rats.

[b] Normal vs vinyl chloride-exposed, $p < 0.05$.

[c] Air control vs vinyl chloride-exposed, $p < 0.05$.

[d] Vinyl chloride (6 weeks) exposed vs vinyl chloride (2 and 4 weeks) exposed, $p < 0.05$.

was signific
6 weeks of e
1). No differ
mixed-functi
serum clinic
exposure, th
weight but t
did not (Tab
the normal c
imal care c
more weight
vinyl chlorid
of exposure,
posed group
mal control
control grou

Morpholo
hedral hepa
plates interp
livers of the
toarchitectu
chloride ex
rats contain
cleus, nume
of rough e
some aggr
ticulum (SE

Duration (week)	
2	
4	
6	

[a] Analysis o
the regression
[b] Normal cc
[c] Air contro
[d] Air contro

was significantly lower than controls after 6 weeks of exposure to vinyl chloride (Table 1). No differences were found in the hepatic mixed-function oxidase activity or in the serum clinical liver tests. After 2 weeks of exposure, the two control groups had gained weight but the vinyl chloride-exposed group did not (Table 2). After 4 weeks of exposure, the normal control group housed at the animal care center had gained significantly more weight than either the air-control or vinyl chloride-exposed group. After 6 weeks of exposure, however, the vinyl chloride-exposed group failed to gain weight; the normal control group gained more than the air-control group (Table 2).

Morphological examination revealed polyhedral hepatocytes arranged in irregular plates interposed by vascular sinusoids in the livers of the control rats. This general cytoarchitecture was maintained after vinyl chloride exposure. Hepatocytes in control rats contained a prominent spherical nucleus, numerous ovoid mitochondria, stacks of rough endoplasmic reticulum (RER), some aggregates of smooth endoplasmic reticulum (SER), and varying amounts of lysosomes and glycogen particles (Fig. 2a). Few interstitial cells were scattered among the hepatocytes. These cells contained few cytoplasmic organelles and could be readily discerned at the light microscopic level by their hyperchromatic nuclei. The sinusoids were linked by fenestrated endothelium and some of the lining cells displayed phagocytic activity. After 2 to 6 weeks of vinyl chloride exposure, the principal organelle affected appeared to be the endoplasmic reticulum. Cisternae of the RER became dilated in a relatively small population of the hepatocytes in the 2-week treatment group. Four weeks after exposure to vinyl chloride, patches of dilated endoplasmic reticulum were prominently displayed in some hepatocytes (Fig. 2b). At this stage the SER was relatively unaffected. In the 6-week treatment group, vesiculation of SER and distention of RER were easily discernible in a large number of hepatocytes (Fig. 2c). However, other cell organelles showed no demonstrable change. These changes, though, are still beyond the resolving limit of the light microscope. The nonhepatocyte components showed minimum changes which

TABLE 2

BODY WEIGHTS OF RATS BEFORE AND AFTER VINYL CHLORIDE EXPOSURE[a]

Duration (week)	Treatment	Initial weight (g)	Final weight (g)	Percentage gain
2	Normal control	400 ± 15	433 ± 16	8[b]
	VC-exposed	405 ± 12	396 ± 14	−2[b,c]
	Air control	396 ± 17	414 ± 18	5[c]
4	Normal control	398 ± 8	450 ± 15	13[b,d]
	VC-exposed	410 ± 16	421 ± 15	3[b]
	Air control	395 ± 10	419 ± 5	6[d]
6	Normal control	402 ± 14	486 ± 14	21[b,d]
	VC-exposed	396 ± 9	398 ± 10	<1[b,c]
	Air control	398 ± 14	449 ± 4	13[c,d]

[a] Rats were exposed to 28,000 ppm of vinyl chloride; normal controls and the air controls were exposed to air only. Each number represents the mean and the SEM from a group of six rats.

[b] Normal vs vinyl chloride-exposed, $p < 0.05$.

[c] Air control vs vinyl chloride-exposed, $p < 0.05$.

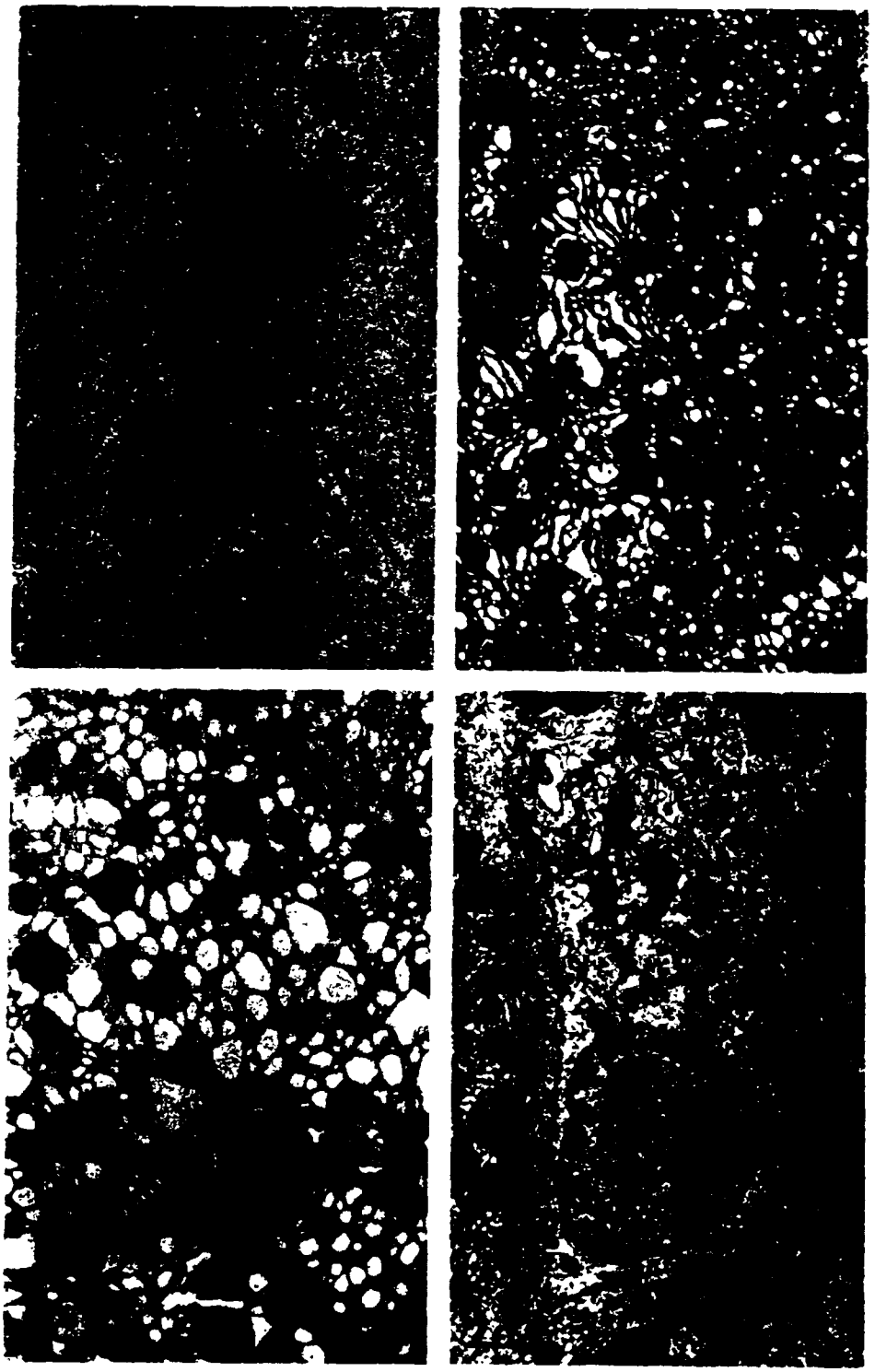
[d] Vinyl chloride (6 weeks) exposed vs vinyl chloride (2 and 4 weeks) exposed, $p < 0.05$.

[a] Analysis of body weight was by regression analysis followed by an analysis of variance on the residuals from the regression equation. (Residual = observed final weight − predicted final weight from regression equation.)

[b] Normal control vs vinyl chloride exposed, $p < 0.05$.

[c] Air control vs vinyl chloride exposed, $p < 0.05$.

[d] Air control vs normal control, $p < 0.05$.



were cl
mulatio
some of
a great
the inte

Gluta
pathwa
harmfu
biotics.
indicat
activati
Gluta
cytosol
glutathi
form les
jugates
posure
could b
outcom
the lon
hepatic
later in
suggest
posures
diate is
the late
chloroo
yield m
react w
ferases
chloroa
sive ch
neously
oxidize
turn m

F
sepa
(b)
two
chlo
betv
(Gl
in a
irre

were characterized by an increased accumulation of lysosomal-like substances in some of the sinusoidal lining cells as well as a greater tendency to accumulate lipids in the interstitial cells (Fig. 2d).

DISCUSSION

Glutathione conjugation is an important pathway for the metabolism of potentially harmful electrophilic metabolites of xenobiotics. Studies by Watanabe *et al.* (1976b,c) indicate this to be the major route for inactivation of the vinyl chloride metabolites.

Glutathione-*S*-transferases are a group of cytosol enzymes catalyzing the reaction of glutathione and electrophilic compounds to form less toxic and more water-soluble conjugates. Their activity during chronic exposure to xenobiotics, like vinyl chloride, could be a key determinate in the ultimate outcome of such exposures as illustrated by the longer arrow in Fig. 1. The increase in hepatic GEST activity at 4 weeks and the later increase in GAST activity at 6 weeks suggested that in the earlier stages of exposures most of the chlorooxirane intermediate is being adequately detoxified. During the later stages of chronic exposure, more chlorooxirane may become rearranged to yield more chloroacetaldehyde and, in turn, react with other available glutathione transferases or become further metabolized to chloroacetic acid. Alternatively, the excessive chlorooxirane could rearrange spontaneously to form chloroethanol and be further oxidized to chloroacetaldehyde, which in turn may react with glutathione, or be oxidized to monochloroacetic acid (Johnson, 1967). This would be consistent with the later increases in the aralkyl-transferases and the finding by Hefner *et al.* (1975) that monochloroacetic acid is found only in the urine of rats exposed for an extended time to higher levels (5000 ppm) of vinyl chloride. The increased use of alternate pathways, for chlorooxirane and chloroacetaldehyde detoxification, may reflect increased concentration of these active metabolites allowing greater opportunity for DNA injury.

A single exposure to vinyl chloride decreased hepatic nonprotein sulfhydryl compounds in rats (Watanabe, 1976a); similar decreases of glutathione concentrations were produced in rats by other xenobiotics such as 1,1-dichloroethylene (Jaeger *et al.*, 1974a; Reichert *et al.*, 1978) and acetaminophen (Mitchell *et al.*, 1973). In the present study, repeated exposure to vinyl chloride caused a significant increase of nonprotein sulfhydryl concentrations (Table 1) analogous to the elevation of glutathione concentrations seen after the administration of carcinogens to rats (Fiala *et al.*, 1976). In addition, the results showed that repeated exposure to vinyl chloride also caused an increase in hepatic glutathione-*S*-transferase activity (Table 1) similar to that seen after the administration of phenobarbital and 3-methylcholanthrene to rats (Mukhtar and Bresnick, 1976). These data suggest a mechanism for compensatory synthesis of hepatic glutathione and glutathione-*S*-transferases after repeated exposure to vinyl chloride.

The decreased concentration of cytochromes *P*-450 found in rats after repeated exposure to vinyl chloride (Table 1) is con-

FIG. 2. (a) Portion of a hepatocyte from control. Stacks of rough endoplasmic reticulum (RER) are separated by many ovoid mitochondria (M). Chromatin is finely dispersed in the nucleus (N). 9300×. (b) Hepatocyte after 2 weeks of vinyl chloride exposure. Dilation of RER appeared widespread in these two cells. Bile (B) canaliculus appeared unaltered in these rats. 5300×. (c) Four weeks after vinyl chloride exposure. Golgi complex (G) appeared unaffected while cisternal dilation continued. Distinction between SER and RER is complicated by the detachment of ribosomes. Lipid droplets (L) and glycogen (GL) often accumulated. 9500×. (d) A fat-storing interstitial cell is surrounded by several hepatocytes in a vinyl chloride-treated animal. Unlike lipid stored in hepatocytes, the shape of lipids (L) appeared irregular in these cells. 8000×.

sistent with work by Reynolds *et al.* (1975). This decrease in cytochromes *P*-450 content was also shown *in vitro* (Guengerich and Strickland, 1977; Ivanetich *et al.*, 1977) suggesting that a metabolite of vinyl chloride destroys the cytochrome. Mixed-function oxidase activity, with benzphetamine as substrate was unaltered.

With regard to the structural alterations produced by vinyl chloride, the present findings confirmed previous observations on the selective effect of this carcinogen in the endoplasmic reticulum (Du *et al.*, 1979). A gradual increase in the number of hepatocytes affected and the involvement of both smooth and rough ER were shown in this study. The endoplasmic reticulum is the primary site of protein synthesis and its dilation suggested the presence of a vinyl chloride-related effect. The lack of a concomitant Golgi hypertropy is noteworthy since this organelle serves as the site of glycosylation and packaging of many exportable proteins. It may be inferred that the vinyl chloride effect is mainly an intracellular phenomenon. The relatively late involvement of SER could reflect a further attempt at detoxification by the hepatocyte. The tendency for the interstitial cells to accumulate lipid and the heightened phagocytic activity correlate with increases in collagen formation and may be evidence of low-grade cellular injury. This may prove to be the initial histological response to vinyl chloride exposure.

Ultrastructural responses to chronic inhalation of vinyl chloride were reported by Feron *et al.* (1979). Unlike our findings, the principal effects observed were swollen mitochondria and some proliferation of SER. The mitochondrial swelling presumably reflects the extensive vacuolization observed by light microscopy. The discrepancy probably resulted from differences in the dose and duration in vinyl chloride exposure since Feron *et al.* (1979) exposed rats to 5000 ppm vinyl chloride for 52 weeks. Such an extensive mitochondrial lesion could result in biochemical modifications such as a change in ATPase or cytochrome oxidase levels. The only enzyme activity measured, glucose-6-phosphatase, was reduced.

The authors believed that the altered glutathione metabolism, as reflected by the increased nonprotein sulfhydryl content, and the increased activities of glutathione-*S*-transferases and glutathione reductase, in rat liver after repeated exposure to high doses of vinyl chloride represent an early hepatocellular adaptation to vinyl chloride exposure.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to the staff of the B. F. Goodrich Plant in Louisville, Kentucky, for their cooperation in the exposure studies, to Dr. Richard A. Greenberg for his review, to Mr. John P. Sandoz for his help with the statistical analysis, and Mr. John Kreisle and Ms. Debra S. Eades for technical assistance.

REFERENCES

BARBIN, A., BRESIL, H., CROISY, A., JACQUIGNON, P., MALAVEILLE, C., MONTESANO, R., AND BARTSCH, H. (1975). Liver-microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.* **67**, 596–603.

BOLT, H. M., KAPPUS, H., BUCHTER, A., AND BOLT, W. (1975). Metabolism of vinyl chloride. *Lancet* **1**, 1425.

BOLT, H. M., KAPPUS, H., KAUFMANN, R., APPEL, K. E., BUCHTER, A., AND BOLT, W. (1976). Metabolism of ^{14}C-vinyl chloride *in vitro* and *in vivo*. *Inserm Symposia Ser.* **52**, 151–164, IARC Scientific Publications No. 13.

CARLBERG, I., AND MANNERVIK, B. (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J. Biol. Chem.* **250**, 5475–5480.

CREECH, J. L., AND JOHNSON, M. N. (1974). Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J. Occup. Med.* **16**, 150–151.

DREW, R. T., HARPER, C., GUPTA, B. N., AND TALLEY, F. A. (1975). Effects of vinyl chloride exposures to rats pretreated with phenobarbital. *Environ. Health Perspec.* **11**, 235–242.

DU, J. T., AND TAMBURRO, C. H. (1976). Decreased glucose-6-phosphatase activity in liver in vinyl chloride exposed rats. *Fed. Proc.* **35**, 329.

xidase levels. The
asured, glucose-6-

at the altered glu-
eflected by the in-
dryl content, and
of glutathione-*S*-
one reductase, in
exposure to high
epresent an early
to vinyl chloride

GMENTS

their gratitude to the
in Louisville, Kentucky,
posure studies, to Dr.
review, to Mr. John P.
istical analysis, and Mr.
Eades for technical as-

CES

Y, A., JACQUIGNON, P., NO, R., AND BARTSCH, -mediated formation of bromide and vinyl chlo- *Commun.* **67**, 596–603.
CHTER, A., AND BOLT, inyl chloride. *Lancet* **1**,

JFMANN, R., APPEL, K. W. (1976). Metabolism nd *in vivo*. *Inserm Sym-* Scientific Publications

IK, B. (1975). Purifica- the flavoenzyme gluta- er. *J. Biol. Chem.* **250**,

M. N. (1974). Angios- icture of polyvinyl chlo- –151.
TA, B. N., AND TALLEY, l chloride exposures to rbital. *Environ. Health*

. H. (1976). Decreased y in liver in vinyl chlo- 35, 329.

DU, J. T., AND TAMBURRO, C. H. (1978). Elevated glutathione content, glutathione-*S*-transferase and glutathione reductase in liver of rats exposed to vinyl chloride. *Fed. Proc.* **37**, 1545.

DU, J. T., SANDOZ, J. P., TSENG, M T., AND TAMBURRO, C. H. (1979). Biochemical alterations in livers of rats exposed to vinyl chloride *J Toxicol Environ. Health* **5**, 1119–1132

ELMORE, J. D., WONG, J. L., LAUMBACH, A. D., AND STREIPS, U. N. (1976). Vinyl chloride mutagenicity via the metabolites chlorooxirane and chloroacetaldehyde monomer hydrate. *Biochim. Biophys Acta* **442**, 405–419.

FERON, V. J., SPIT, B. J., IMMEL, H. R., AND KROES, R. (1979). One-year time sequence inhalation toxicity study of vinyl chloride in rats. III. Morphological changes in the liver. *Toxicology* **13**, 143–154.

FIALA, S., MOHINDRU, A., KETTERING, W. G., FIALA, A. E., AND MORRIS, H. P. (1976). Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. *J. Nat. Cancer Inst.* **57**, 591–598.

GREEN, T., AND HATHWAY, D. E. (1975). The biological fate in rats of vinyl chloride in relation to its oncogenicity. *Chem.–Biol. Interact.* **11**, 545–562.

GREEN, T., AND HATHWAY, D. E. (1977). The chemistry and biogenesis of S-containing metabolites of vinyl chloride in rats. *Chem.–Biol. Interact.* **17**, 137–150.

GREIM, H., BONSE, G., RADWAN, Z., REICHERT, D., AND HENSCHLER, D. (1975). Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* **24**, 2013–2017.

GROSS, H., AND FREIBERG, J. (1969). Alpha-halo ethers. 41. Existence of chloroethylene oxide. *J. Prakt. Chem.* **311**, 506–510.

GUENGERICH, F. P., AND STRICKLAND, T. W. (1977). Metabolism of vinyl chloride: Destruction of the heme of highly purified liver microsomal cytochrome P-450 by a metabolite. *Mol. Pharmacol.* **13**, 993–1004.

HABIG, W. H., PABST, M. J., AND JAKOBY, W. B. (1974). Glutathione-*S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol. Chem.* **249**, 7130–7139.

HEFNER, R. E., WATANABE, P. G., AND GEHRING, P. J. (1975). Preliminary studies of the fate of inhaled vinyl chloride monomer in rats. *Ann. N.Y. Acad. Sci.* **246**, 135–148.

IVANETICH, K. M., ARONSON, I., AND KATZ, I. D. (1977). The interaction of vinyl chloride with rat hepatic microsomal cytochrome P-450 *in vitro*. *Biochem. Biophys. Res. Commun.* **74**, 1411–1418.

JAEGER, R. J., CONNOLLY, R. B., AND MURPHY, S. D. (1974a). Effect of 18 hr. fast and glutathione depletion on 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats. *Exp. Mol Pathol.* **20**, 187–198.

JAEGER, R. J., REYNOLDS, E. S., CONNOLLY, R. B., MOSLEN, M. T., SZABO, S., AND MURPHY, S D. (1974b). Acute hepatic injury by vinyl chloride in rats pretreated with phenobarbital. *Nature (London)* **252**, 724–726.

JOHNSON, M. K. (1967). Metabolism of chloroethanol in the rat. *Biochem. Pharmacol.* **16**, 185–199.

KAPLOWITZ, N., KUHLENKAMP, J., AND CLIFTON, G. (1975). Drug induction of hepatic glutathione-*S*-transferases in male and female rats. *Biochem. J.* **146**, 351–356.

KAPPUS, H., BOLT, H. M., BUCHTER, A., AND BOLT, W. (1976). Liver microsomal uptake of ^{14}C-vinyl chloride and transformation to protein alkylating metabolites *in vitro*. *Toxicol. Appl. Pharmacol.* **37**, 461–471.

LAIB, R. J., AND BOLT, H. M. (1978). Formation of 3, N^4-ethenocytidine moieties in RNA by vinyl chloride metabolites *in vitro* and *in vivo*. *Arch. Toxicol.* **39**, 235–240.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

LU, A. Y. H., KUNZTZMAN, R., WEST, S., JACOBSON, M., AND CONNEY, A. H. (1972). Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and exogenous substrates. *J. Biol. Chem.* **247**, 1727–1734.

MALAVEILLE, C., BARTSCH, H., BARBIN, A., CAMUS, A. M., AND MONTESANO, R. (1975). Mutagenicity of vinyl chloride, chloroethylene oxide, chloroacetaldehyde and chloroethanol. *Biochem. Biophys. Res. Commun.* **63**, 363–370.

MALTONI, C., AND LEFEMINE, C. (1975). Carcinogenicity bioassays of vinyl chloride: Current results. *Ann. N.Y. Acad. Sci.* **246**, 195–218.

MCCANN, J., SIMMON, V., STREITWIESER, D., AND AMES, B. N. (1975). mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane, chloroethanol, vinyl chloride and cyclophosphamide. *Proc. Nat. Acad. Sci. USA* **72**, 3190–3193.

MITCHELL, J. R., JOLLOW, D. J., POTTER, W. A., GILLETE, J. R., AND BRODIE, B. B. (1973). Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.* **187**, 211–217.

MUKHTAR, H., AND BRESNICK, E. (1976). Effects of phenobarbital and 3-methylcholanthrene administration on glutathione-*S*-epoxide transferase activity in rat liver. *Biochem. Pharmacol.* **25**, 1081–1084.

OMURA, T., AND SATO, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**, 2370–2378.

REICHERT, D., WERNER, H. W., AND HENSCHLER, D.

(1978). Role of liver glutathione in 1,1-dichloroethylene metabolism and hepatotoxicity in intact rats and isolated perfused rat liver. *Arch. Toxicol.* **41,** 169–178.

REYNOLDS, E. S., MOSLEN, M. T., SZABO, S., AND JAEGER, R. J. (1975). Vinyl chloride-induced deactivation of cytochrome P-450 and other components of the liver mixed function oxidase system: An *in vivo* study. *Res. Commun. Chem. Pathol. Pharmacol.* **12,** 685–694.

SEDLAK, J., AND LINDSAY, R. H. (1968). Estimation of total protein bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **25,** 192–205.

VANDUUREN, B. L. (1975). Possible mechanism of carcinogenic action of vinyl chloride. *Ann. N.Y. Acad. Sci.* **246,** 258–267.

VIOLA, P. L., BIGOTTI, A., AND CAPUTO, A. (1971). Oncogenic response of rat skin, lungs and bones to vinyl chloride. *Cancer Res.* **31,** 516–522.

WATANABE, P. G., HEFNER, R. E., JR., AND GEHRING, P. J. (1976a). Vinyl chloride-induced depression of hepatic non-protein sulfhydryl content and effects of bromosulphalein (BSP) clearance in rats. *Toxicology* **6,** 1–8.

WATANABE, P. G., MCGOWAN, G. R., AND GEHRING, P. J. (1976b). Fate of ^{14}C-vinyl chloride after single oral administration in rats. *Toxicol. Appl. Pharmacol.* **36,** 339–352.

WATANABE, P. G., MCGOWAN, G. R., MADRID, E. O., AND GEHRING, P. J. (1976c). Fate of ^{14}C-vinyl chloride following inhalation exposure in rats. *Toxicol. Appl. Pharmacol.* **37,** 49–50.

WATANABE, P. G., ZEMPEL, J. A., PEGG, D. G., AND GEHRING, P. J. (1978). Hepatic macromolecular binding following exposure to vinyl chloride. *Toxicol. Appl. Pharmacol.* **44,** 571–579.