

BIOCHEMICAL ALTERATIONS IN LIVERS OF RATS EXPOSED TO VINYL CHLORIDE

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Sprague-Dawley rats were exposed to vinyl chloride to determine the earliest sequential biochemical changes occurring with liver injury before angiosarcoma development. Activity of glucose-6-phosphatase, a key gluconeogenic enzyme in the liver microsomal fraction, decreased 25% with respect to controls after 70 h of exposure. Glucose-6-phosphate dehydrogenase activity increased twofold after more than 100 h of exposure. Nonprotein sulphydryl levels (glutathione and/or cysteine) showed a slight but progressive elevation, whereas glutathione reductase activity increased 50-60% during exposure to vinyl chloride. NADPH-cytochrome c reductase and mixed function oxidase were unchanged in the same microsomal fraction. There were no changes in seven conventional clinical biochemical liver tests or in four other markers of liver mitochondrial, cytosol, and microsomal function. No significant histological changes were found on light microscopic examination during this exposure period. However, with electron microscopy, dilation of rough endoplasmic reticulum was seen in the animals exposed for more than 137 h. These enzymatic changes are considered to reflect early hepatocellular adaptation to vinyl chloride exposure with very mild or limited hepatocellular injury in its earliest stage.

INTRODUCTION

Vinyl chloride has been shown to induce tumors, including angiosarcoma, in laboratory animals (Maltoni and Lefemine, 1975; Viola et al., 1971) and angiosarcoma in humans (Creech and Johnson, 1974). At high concentrations, vinyl chloride is believed to be metabolized by the microsomal mixed function oxidase (MFO) system of the liver to toxic metabolites (Bolt et al., 1975; Hefner et al., 1975; Johnson, 1967; Watanabe et al., 1976b, 1976c). Animals pretreated with phenobarbital, an inducer of

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MFO, and exposed to 5% vinyl chloride (50,000 ppm) had increased serum alanine aminotransferase, a conventional biochemical indicator of liver injury, and increased serum sorbitol dehydrogenase, a liver specific enzyme (Jaeger et al., 1974). However, there have been no sequential enzymatic studies to determine what progressive metabolic alterations of the hepatocyte occur during vinyl chloride exposure preceding the development of angiosarcoma. Although the hepatocyte is the major cell for oxidation and probably detoxification of vinyl chloride and its metabolites, angiosarcoma develops not in the hepatocyte but in adjacent mesenchymal cells. During this time, the hepatocytes undergo changes (focal nodular hyperplasia) suggesting focal regeneration, probably from limited hepatocellular injury (Tamburro et al., 1979).

The purpose of our study was to characterize the earliest sequential enzymatic changes that occurred in vinyl chloride exposure to elucidate the hepatocytes' role in vinyl chloride-induced injury and angiosarcoma development. Portions of this study have been presented elsewhere (Du and Tamburro, 1976).

METHODS

Animals and Experimental Design

Sprague-Dawley male rats (300-500 g) were randomly assigned before each experiment to either a group to be exposed to vinyl chloride gas (15,000 ppm) or a nonexposed control group kept outside the chamber in the animal room. Animals were fed standard laboratory chow pellets *ad libitum*.

Four sequential experiments were done. In experiments A and B, rats were exposed 2-4 h/d, 4 d/wk, for 1-2 wk with total accumulated exposure times of 14, 28, and 42 h. In experiment C, rats were exposed 4-8 h/d, 5 d/wk, for 2-3 wk with total accumulated exposure periods of 71 and 103 h. In experiment D, rats were exposed 6-8 h/d, 5 d/wk, for 3-4 wk with total accumulated exposure periods of 84 and 137 h.

The animals were exposed in a modified chamber consisting of an airtight vat that had been used for manufacturing polyvinyl chloride. An aliquot of vinyl chloride (185 g) was added to the vat per 4-d period to make an average concentration of $15,000 \pm 4000$ ppm. The air was constantly circulated by a stirrer. The chamber volume was 4400 l (1100 gal), so the respiration of the rats had a negligible effect on the composition of the chamber's atmosphere. Experiments with an added control group exposed only to air in an identical modified vat showed no differences from the controls kept in the animal room (Table 1).

All animals were anesthetized with ether, had blood drawn by cardiac puncture, and were sacrificed at the same time of day (within 3 h after final exposure) to avoid diurnal effects.

TABLE 1. Comparison of Hepatic Enzyme Activities in Animal Control Groups Based in Animal Quarters and in the Exposure Chamber^a

Enzyme	Air exposed	
	Animal room	Chamber ^b
Glucose-6-phosphate dehydrogenase	12.82 ± 1.32 ^c	11.06 ± 1.78 ^c
Glutathione reductase	4.77 ± 0.27 ^d	5.28 ± 0.40 ^d

^a Values are means ± SEM (*n* = 6).^b Kept in chamber 7 h/d, 5 d/wk, for 6 wk; total, 210 h.^c Not significant.^d Not significant.

Sample Preparation

Liver was excised rapidly and was immediately rinsed in ice-cold 0.15 M KCl with 0.02 M Tris buffer, pH 7.4. A portion of the tissue was homogenized with 9 volumes of the cold KCl-Tris buffer in a Potter-Elvehjem homogenizer. Each sample was prepared from a single organ and kept at 4°C during preparation. Remaining liver was frozen rapidly and stored at -20°C. For assays with frozen tissue, livers from control and experimental rats were frozen in an identical manner for the same length of time.

Subcellular Fractionation and Biochemical Determination

Homogenate was centrifuged at 600 and 8000 X *g* for 10 min and at 100,000 X *g* for 60 min to obtain the nuclear, mitochondrial, microsomal, and cytosol fractions, respectively, by the differential technique of Schneider and Hogeboom (1950). Centrifugation was at 4°C in a Sorvall refrigerated RCS supercentrifuge with a fixed angle rotor and a Beckman model L-5 ultracentrifuge with a swinging bucket rotor. Enzyme activities and cytochrome P-450 were determined in the isolated subcellular fractions: cytochrome oxidase in the mitochondrial fraction; NADPH-cytochrome c reductase, mixed function oxidase, cytochrome P-450, and glucose-6-phosphatase in the microsomal fraction; and glutathione reductase and glucose-6-phosphate dehydrogenase in the 100,000 X *g* supernatant fraction.

Cytochrome P-450, NADPH-cytochrome c reductase, and cytochrome oxidase were determined in fresh samples; all other determinations were in freshly isolated fractions from frozen tissue. Glucose-6-phosphatase was estimated by measuring release of inorganic phosphate (Harper, 1965). Cytochrome P-450 was estimated by the maximum absorption difference between the dithionite-reduced cytochrome and its CO complex (Omura and Sato, 1964). The P-450 concentration was calculated by using 91,000

$M^{-1} \text{ cm}^{-1}$ as the extinction coefficient for the increase in peak height between 490 and 450 nm. Cytochrome oxidase was measured as described by Wharton and Tzagoloff (1967), and NADPH-cytochrome *c* reductase according to Degroot and Dunn (1964). Mixed function oxidase was determined according to Holtzman et al. (1968) by measuring the hydroxylation of analine. Nonprotein sulfhydryl was estimated by the method of Sedlak and Lindsay (1968). Rate of oxidation of NADPH by oxidized glutathione was used as a measure of enzymatic activity of glutathione reductase (Carlberg and Mannervik, 1975). Glucose-6-phosphate dehydrogenase was measured spectrophotometrically, as the rate of NADPH formation (Lohr and Waller, 1965). Assays were conducted under conditions of linearity with respect to both time and protein. Protein content was determined by the method of Lowry et al. (1951). Conventional clinical analyses of serum included aspartate aminotransferase (AST, SGOT), alanine aminotransferase (ALT, SGPT), alkaline phosphatase, bilirubin, albumin, cholesterol, and triglyceride, determined by the technicon sequential multiple analyzer computer (SMAC) system.

Materials

The NADPH, cytochrome *c*, and oxidized glutathione were obtained from Sigma Chemical Co., St. Louis, Mo. Analine and other chemicals were reagent grade. Double-distilled water was used throughout.

Light and Electron Microscopy

Small strips of liver were removed under ether anesthesia and immersed immediately in ice-cold 3% glutaraldehyde (pH 7.4). Tissues were sliced into small cubes and fixed for 2 h at 4°C. Subsequently, samples were washed overnight in phosphate buffer and postfixated in 1% osmium tetroxide for 1 h before being dehydrated in ascending alcohol and embedded in Epon. Tissue blocks were polymerized at 60°C for 2 d. 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, control rats and rats exposed for 42 and 137 h to vinyl chloride were reviewed.

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 before examination.

RESULTS

The protein contents (milligrams per gram of liver) of the subcellular fractions in control and vinyl chloride-exposed groups were the same throughout the exposure; therefore, the enzymatic results were expressed as micromoles converted per minute per milligram of subcellular protein.

The statistical analysis (see below) showed certain significant

enzymatic differences between the exposed and control groups in glucose-6-phosphatase, glutathione reductase, and glucose-6-phosphate dehydrogenase.

Glucose-6-phosphatase. No significant differences in glucose-6-phosphatase activity were detected between the two groups up to 42 h; however, after 71 h, the glucose-6-phosphatase activity was significantly less in the exposed than in the control animals. Figure 1a shows the 25% decrease in mean activity of glucose-6-phosphatase after 71 h; this activity remained significantly lower up to 137 h. Figure 1b shows the 95% confidence intervals for D , the mean difference between the exposed group and the control groups, in each experiment.

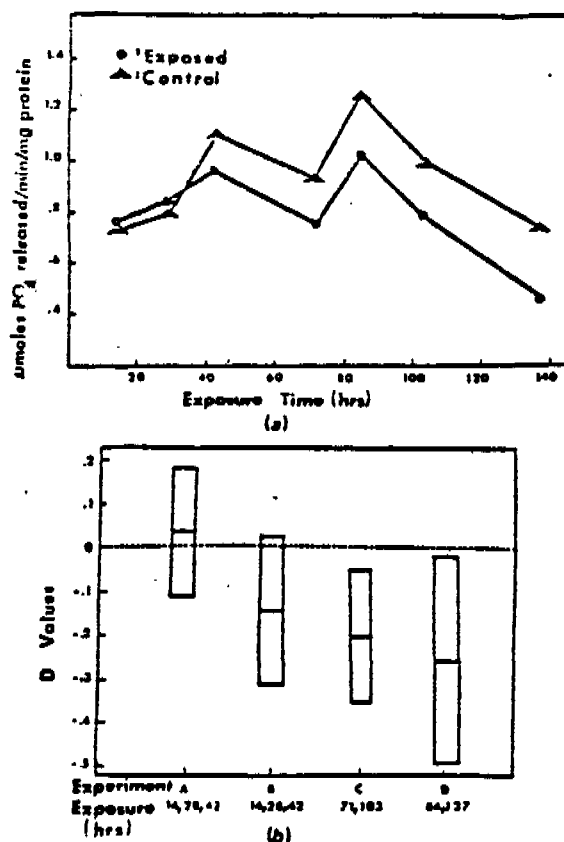


FIGURE 1. (a) Composite curve of group means of specific activity of glucose-6-phosphatase with respect to exposure time; (b) 95% confidence intervals for D , the mean difference between the exposed and control groups. There is no significant difference between the exposed and control groups until after 42 h of exposure. After 71 h the mean level of the exposed group is significantly less than that of the control group.

Glutathione Reductase. Figure 2, *a* and *b*, illustrates the significant differences ($p < 0.05$) between the exposed and control groups in all 4 experiments except at 71 h of exposure ($p < 0.06$). The glutathione reductase level was 25% greater in exposed than in nonexposed animals up to 71 h and approximately 50% greater after 84 and 137 h.

Glucose-6-phosphate Dehydrogenase. There was no significant difference between exposed and control groups from 14 to 42 h and at 71 and 84 h. However, after 103 and 137 h of exposure there was a statistically significant difference as shown in Fig. 3, *a* and *b*. After about 100 h of exposure, the mean glucose-6-phosphate dehydrogenase level in exposed animals was about twice that in nonexposed animals.

While these differences were occurring, none of the conventional biochemical liver function tests (e.g., serum aminotransferases, alkaline phosphatase) showed any significant change, nor were there any changes in cytochrome oxidase, mixed function oxidase, P-450, or NADPH-cytochrome *c* reductase.

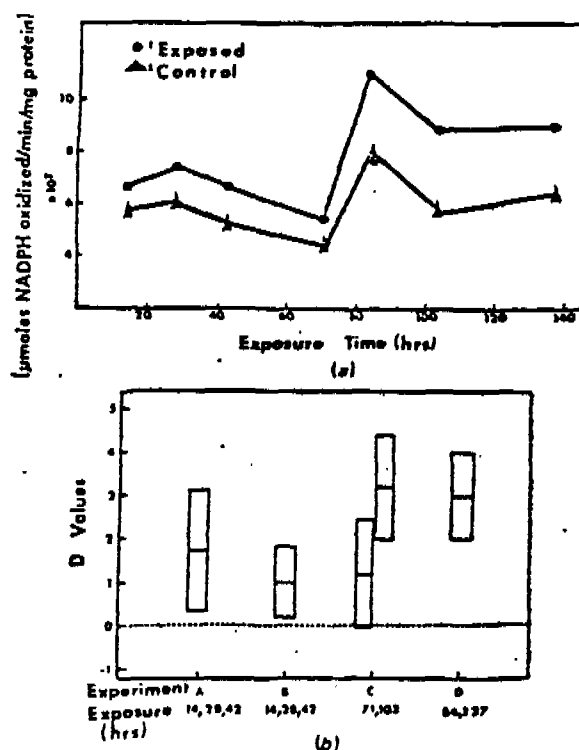


FIGURE 2. (a) Composite curve of group means of specific activity of glutathione reductase with respect to exposure time; (b) 95% confidence intervals for *D*, the mean difference between the exposed and control group. The mean value for the exposed group is significantly greater than that for the controls throughout the entire experiment ($p < 0.05$) except at 71 h of exposure.

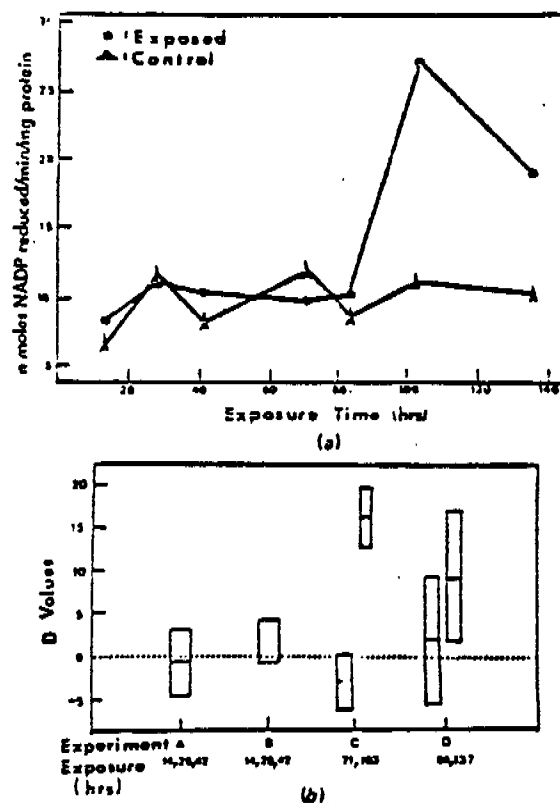


FIGURE 3. (a) Composite curve of group means of specific activity of glucose-6-phosphate dehydrogenase with respect to exposure time; (b) 95% confidence intervals for D , the mean difference between the exposed and control groups. The mean value for the exposed group is not significantly different from that for the control group until after 84 h of exposure.

The concentration of reduced glutathione in the livers of rats decreased to 52% of the control value after a single 2-h exposure to 15,000 ppm vinyl chloride (Table 2). However, the reduced glutathione concentration appeared to be the same or slightly elevated when the rats underwent multiple exposures. This elevation was not significant at the 5% level. The vinyl chloride-exposed rats suffered a weight loss of 4-13% and the control group gained 2-10% (Table 3). All rats survived to the termination of the experiment without noticeable ill effects.

Statistical Analysis of Results

The 95% confidence interval for group mean differences in each enzyme study was based on the error mean square from a two-factor analysis of variance with interaction, the two main effects being exposure time in hours and exposure-nonexposure. This analysis was performed for

TABLE 2. Effect of Vinyl Chloride Exposure on Concentration of Liver Nonprotein Sulfhydryl Compound in Rats

Exposure time (h)	Concentration ratio (exposed/control)
2 ^a	0.52 (3) ^b
14 ^c	1.02 (6)
28	0.96 (6)
42	0.82 (6)
71	1.21 (5)
84	1.07 (3)
103	1.49 (6)
137	1.29 (3)

^aSingle exposure.^bNumber of animals in control or experimental group is shown in parentheses.^cMultiple exposures (4-8 h/d, 4-5 d/wk) for 14-137 h.TABLE 3. Body Weights of Rats before and after Vinyl Chloride Exposure^a

Accumulated exposure (h)	Time	Control	Change (%)	Vinyl chloride-exposed	Change (%)
14	Before	442 ± 6.2 ^b (3) ^c	+3	453 ± 1.5 (3)	-7
	After	454 ± 9.5 (3)		421 ± 4.1 (3)	
28	Before	436 ± 9.2 (3)	+2	448 ± 6.0 (3)	-9
	After	445 ± 12.1 (3)		408 ± 3.0 (3)	
42	Before	433 ± 5.2 (3)	+5	450 ± 4.3 (3)	-9
	After	455 ± 6.4 (3)		410 ± 2.8 (3)	
71	Before	437.6 ± 12.2 (5)	+2	422.7 ± 9.2 (5)	-13
	After	447 ± 19.4 (5)		385.0 ± 4.2 (5)	
84	Before	412.7 ± 7.9 (3)	+10	425 ± 17.4 (3)	-6
	After	455.0 ± 6.8 (3)		399 ± 22 (3)	
103	Before	453 ± 12.9 (6)	+3	441.5 ± 6.8 (6)	-10
	After	465 ± 16.9 (6)		397.0 ± 5.7 (6)	
137	Before	404.7 ± 1.2 (3)	+5	415.0 ± 15.7 (3)	-4
	After	425.0 ± 5.2 (3)		398 ± 28 (3)	

^aMultiple exposures to 1.5% vinyl chloride for 14-137 h, 4-8 h/d, 4-5 d/wk.^bResults are expressed as mean ± SEM.^cNumber of animals is given in parentheses.

each of the four experiments. Overall significant ($p < 0.05$) differences between the exposed and control groups are found when the 95% confidence intervals (D values) *do not* contain zero.

In Fig. 1*b*, there is no significant difference ($p > 0.05$) between the 2 groups in experiments A and B because a D value of zero is within these two confidence intervals. However, for experiments C and D, the confidence intervals do not contain a D value of zero, indicating a significant difference ($p < 0.05$) between the exposed and control groups. When the D value is less than zero (as in Fig. 1*b*), the experimental group is significantly lower than the control throughout the experiment; when the D value is greater than zero (as in Figs. 2 and 3), the experimental group is significantly higher than the control. Further, in experiments C and D of Fig. 1*b*, a single confidence interval is shown even though there are two time periods of exposure each. This results from the nonsignificance of the interaction term in the analysis of variance, meaning that the observed difference at 71 h of exposure is not statistically different from the difference at 103 h. Similar conclusions hold for experiment D in Fig. 1*b*. Figure 2*b* shows 2 confidence intervals for the set of experiments C at 71 and 103 h because the interaction of the time and exposure factors was significant; that is, the difference between the exposed and control groups after 103 h of exposure was significantly greater than the difference after 71 h. This is also reflected by the differences of the mean values at 71 and 103 h as shown in Fig. 2*a*. For the same reasons, there are also two confidence intervals for the C (71 and 103 h) and D (84 and 137 h) sets of experiments in Fig. 3*b*.

Morphological Studies

Morphological light microscopic studies were performed in a blind (coded) and randomized fashion and did not show any evidence of hepatocellular injury or changes usually seen in the latter stages of vinyl chloride exposure.

Besides some variations in glycogen content, no difference in the fine structure of the hepatocytes was observed between controls (Fig. 4) and those exposed to vinyl chloride for 42 h. Dilation of rough endoplasmic reticulum was observed in a small number of hepatocytes after 137 h of vinyl chloride exposure (Fig. 5). Although no noticeable change in the amount of smooth endoplasmic reticulum was associated with this change, a concomitant increase in cytoplasmic density was evident in these cells. Another type of lesion found in *other* hepatocytes was characterized by the presence of small patches of clear spaces, which tended to aggregate near the cell periphery (Fig. 6). Such lesions usually affect the adjacent cell equally. No other cell type appeared to be affected by vinyl chloride exposure in this study.

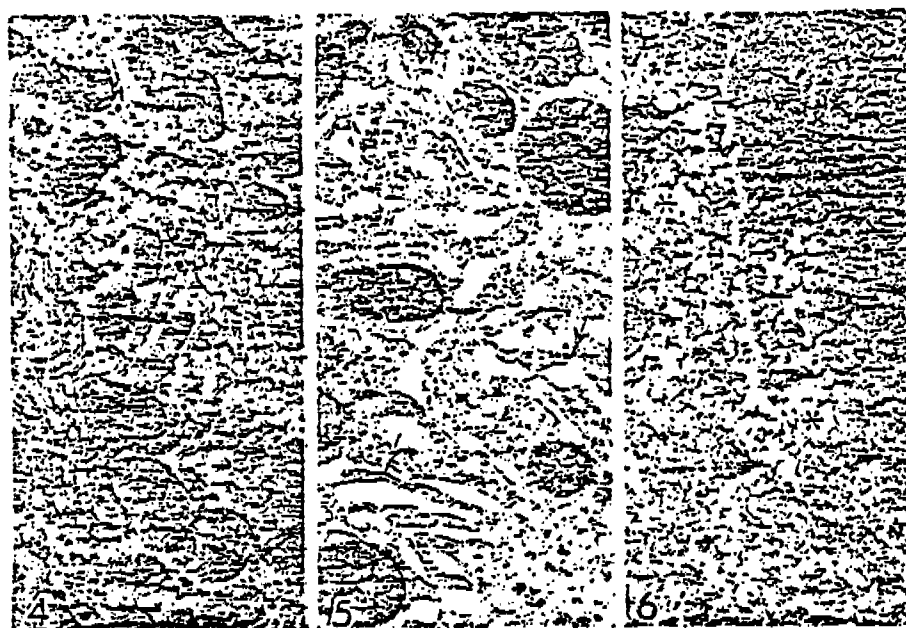


FIGURE 4. Portion of a hepatocyte from a control rat. A good complement of mitochondria and rough endoplasmic reticulum is shown (X12,000).

FIGURE 5. Dilation of RER (arrows) shown in hepatocyte 137 h after exposure to 15,000 ppm vinyl chloride during 2-3 wk (X25,000).

FIGURE 6. Subplasmalemmal lesions (*) in hepatocytes 137 h after exposure to 15,000 ppm vinyl chloride during 2-3 wk. The lesions are present in two adjacent cells and the clear spaces seem to coalesce (X12,000).

DISCUSSION

Decreased glucose-6-phosphatase activity and increased glucose-6-phosphate dehydrogenase and glutathione reductase activity in rat liver after exposure to vinyl chloride (as in our studies) may be the biochemical alterations indicative of early liver injury, adaptation to increased detoxification activity, or preparation for increased nucleic acid synthesis. These differences were observed before any abnormalities were detectable from conventional liver function tests, such as serum aminotransferases, or from changes in other subcellular organelle markers, such as mitochondrial cytochrome oxidase activity.

In primary hepatocellular cancer (hepatomas), the activity of key enzymes for gluconeogenesis (glucose-6-phosphatase, etc.) decreased with increased rate of tumor growth (Weber, 1974; Weber and Convery, 1966; Weber and Lea, 1967). In contrast, two enzymes for the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (Selmecki and

Weber, 1976; Weber and Morris, 1963) and transaldolase (Heinrich et al., 1974), increased in all hepatomas. Further, the activity of glucose-6-phosphatase decreased before and during the development of hepatomas when carcinogens such as nitrosamine and dimethylaminoazobenzene were fed to rats (Isok and Teras, 1973; Weber and Cantero, 1955). Whether our similar findings in this study are indicators of eventual cancer development (angiosarcoma) is not yet known. We used a shorter exposure period in order to identify the biochemical changes that would best reflect the morphological and cellular changes anticipated on the basis of previous human and animal studies. No attempt was made to determine what effects these shorter exposure periods would induce with long-term observation. This is now in progress. However, Maltoni and Lefamine (1975) showed that of 69 Sprague-Dawley rats exposed to 10,000 ppm vinyl chloride, 16 (26%) developed Zymbal gland carcinomas after 50 wk, 5 (8%) developed nephroblastomas after 59 wk, and 9 (15%) developed angiosarcomas after 64 wk.

The vinyl chloride-exposed groups lost weight (4-13%) whereas the control group gained weight (2-10%) during the entire experimental period. However, the differences in the three enzymes cannot be accounted for by lack of dietary food intake in the experimental group, because fasting increases glucose-6-phosphatase (Ashmore et al., 1954) and decreases glucose-6-phosphate dehydrogenase (Winberry and Holten, 1977), and we found decreased glutathione reductase and reduced glutathione content in fasted animals (unpublished data).

Rats exposed to a high dose (5%) of vinyl chloride (Reynolds et al., 1975) had decreased mixed function oxidase activity. An *in vitro* study (Ivanetich et al., 1977) showed that the metabolites of vinyl chloride from the microsomal enzyme system decreased the levels of cytochrome P-450. In our studies; the cytochrome P-450 content and the activity of mixed function oxidase and NADPH-cytochrome c reductase in rats repeatedly exposed to 1-2% vinyl chloride did not show any such changes. Subsequent experiments in which rats were exposed to 28,000 ppm vinyl chloride for 70-210 h in 2-6 wk showed a significant decrease of cytochrome P-450 concentration (in preparation). Differences in dose level or experimental design may account for these variations. Drew et al. (1975) found that the activity of mixed function oxidase depended on the time interval between cessation of exposure to vinyl chloride and sacrifice of animals.

The main detoxification route for vinyl chloride metabolism is thought to be conjugation with glutathione (Johnson, 1967); sulfur-containing urinary metabolites of radioactively labeled vinyl chloride have been found in rats' urine (Green and Hathway, 1975, 1977; Watanabe et al., 1976b, 1976c).

We found that under the present experimental conditions the non-protein sulfhydryl content of liver (glutathione and/or cysteine) tended to

increase in rats repeatedly exposed to vinyl chloride (Table 2). Although the difference was not significant in this experiment, subsequent exposure of rats to 28,000 ppm for 70, 140, and 210 h in 2, 4, and 6 wk led to a significant elevation of the nonprotein sulfhydryl content in liver (Du and Tamburro, 1978). Watanabe et al. (1976a) and Johnson (1967) found a depletion of nonprotein sulfhydryl content in rats after only a single exposure to vinyl chloride or chloroethanol. Hefner et al. (1975) found that a single vinyl chloride exposure reduced the glutathione content by one-half, but the decrease became smaller and even unnoticeable after repeated exposure to vinyl chloride. Fiala et al. (1976) showed that the reduced glutathione content increased after prolonged exposure of rats to various chemical carcinogens. These results are thought to reflect the exposed animals' attempt to make more glutathione to meet the unusually strong demand for detoxification.

Glutathione reductase was elevated in our system after rats were exposed to vinyl chloride for 42 h. This enzyme generates reduced glutathione from its oxidized form as a compensatory mechanism to maintain the level of glutathione, and the simultaneous elevation of glucose-6-phosphate dehydrogenase regenerates NADPH, which can be used as a cofactor for various synthetic pathways including nucleic acid synthesis. Elevated glutathione reductase activity was found in rats with primary hepatocellular cancer induced by diethylnitrosamine (Pinto and Bartley, 1973). This consistent increase in glutathione reductase activity after exposure to vinyl chloride suggests that it may be one of the earliest biochemical manifestations of exposure and injury.

These metabolic changes could also play a role in the early changes observed by light and electron microscopy. Hepatic lesions such as dilation of smooth endoplasmic reticulum and loss of microvilli were reported in mice as early as 1 mo after vinyl chloride exposure (Schaffner et al., 1976). In contrast, we observed dilation of rough endoplasmic reticulum and patchy lesions near the plasmalemma. The dilated rough endoplasmic reticulum could be related to the increased enzyme synthesis (i.e., glucose-6-phosphate dehydrogenase and glutathione reductase) induced by vinyl chloride. The absence of smooth endoplasmic reticulum proliferation in our animals suggests a comparatively milder effect in our short-term study. In a subsequent study where long-term effects of vinyl chloride exposure was assessed, dilation of smooth endoplasmic reticulum in hepatocytes was observed (in preparation). The nature of the patchy lesions found near the plasmalemma remains to be established. The location of the lesion, however, suggests that some toxic agents may be entering or exiting the hepatocytes in these sites.

We believe these early enzymatic changes reflect adaptation of the liver cell to early mild injury. The alterations in gluconeogenesis and in the pentose phosphate shunt appear to indicate the liver's adaptation while undergoing repetitive and prolonged exposure to the mildly toxic chemical

vinyl chloride, which, when inadequately metabolized, produces intermediates that lead to the formation of cancer.

REFERENCES

- Ashmore, J., Hastings, A. B., and Nesbett, F. B. 1954. The effect of diabetes and fasting on liver glucose-6-phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* 40:673-678.
- Bolt, H. M., Kappus, H., Buchter, A., and Bolt, W. 1975. Metabolism of vinyl chloride. *Lancet* i:1425.
- Carlberg, I. and Mennervik, 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.
- Degroot, L. J. and Dunn, A. D. 1964. Electron-transport enzymes of calf thyroid. *Biochim. Biophys. Acta* 92:205-222.
- Draw, R. T., Harper, C., Gupta, B. N., and Tatley, F. A. 1975. Effects of vinyl chloride exposures to rats pretreated with phenobarbital. *Environ. Health Perspect.* 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.
- 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.
- Fiala, S., Mohindru, A., Ketterling, W. G., Fiala, A. E., and Morris, H. P. 1976. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. *J. Natl. 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.
- Harper, A. E. 1965. Glucose-6-phosphatase. In *Methods of Enzymatic Analysis*, ed. H. U. Bergmeyer, pp. 788-792. New York: Academic.
- 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.
- Heinrich, P. C., Morris, H. P., and Weber, G. 1974. Increased phosphoribosylpyrophosphate synthetase activity in rapidly growing hepatomas. *FEBS Lett.* 42:145-148.
- Holtzman, J. L., Gram, T. E., Gigon, P. L., and Gillette, J. R. 1968. The distribution of the components of mixed-function oxidase between the rough and the smooth endoplasmic reticulum of liver cells. *Biochem. J.* 110:407-412.
- Isok, M. E. and Teras, L. E. 1973. Glucose-6-phosphatase activity in liver carcinogenesis and in transplantable hepatoma in mice. *Vopr. Med. Khim.* 19:568-570.
- 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., Reynolds, E. S., Connolly, R. B., Moslen, M. T., Szabo, A., and Murphy, S. M. 1974. Acute hepatic injury by vinyl chloride in rats pretreated with phenobarbital. *Nature (Lond.)* 252:724-726.
- Johnson, M. K. 1967. Metabolism of chloroethanol in the rat. *Biochem. Pharmacol.* 16:185-199.
- Lohr, G. W. and Waller, H. D. 1965. Glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis*, ed. H. U. Bergmeyer, pp. 744-751. New York: Academic.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Maltoni, C. and Lafemine, G. 1975. Carcinogenicity bioassays of vinyl chloride: Current results. *Ann. N.Y. Acad. Sci.* 246:195-218.

- 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.
- Pinto, R. E. and Bartley, W. 1973. Glutathione reductase and glutathione peroxidase activities in hepatomas of rats treated with diethyl nitrosamine. *FEBS Lett.* 32:307-309.
- 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.
- Schaffner, F., Popper, H., and Selikoff, I. J. 1976. Initial features of vinyl chloride hepatic injury. *Gastroenterology* 71:A35 (abstr.).
- Schneider, W. C. and Hogeboom, G. H. 1950. Intracellular distribution of enzymes. V. Further studies on the distribution of cytochrome c in rat liver homogenates. *J. Biol. Chem.* 183:123-128.
- Sedlak, J. and Lindsay, R. H. 1968. Estimation of total protein bound and non-protein sulphydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* 25:192-205.
- Selmecki, L. E. and Weber, G. 1976. Increased glucose-6-phosphate dehydrogenase concentration in hepatoma 3924A: Enzymic and immunological evidence. *FEBS Lett.* 61:63-67.
- Tamburro, C. M., Makk, L., and Popper, H. 1979. Early hepatic histological alterations among chemical (vinyl monomer) workers. *Gastroenterology*, in press.
- 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., Helmer, R. E., Jr., and Gehring, P. J. 1976a. Vinyl chloride-induced depression of hepatic non-protein sulphydryl content and effects on bromosulphalein (BSP) clearance in rats. *Toxicology* 6:1-8.
- Watanabe, P. G., McGowan, G. R., and Gehring, P. M. 1976b. Fate of ¹⁴C-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 ¹⁴C-vinyl chloride following inhalation exposure in rats. *Toxicol. Appl. Pharmacol.* 37:49-50.
- Weber, G. 1974. The molecular correlation concept. In *The Molecular Biology of Cancer*, ed. H. Busch, pp. 487-521. New York: Academic.
- Weber, G. and Cantero, A. 1955. Glucose-6-phosphatase activity in normal, precancerous, and neoplastic tissues. *Cancer Res.* 15:105-108.
- Weber, G. and Convery, H. J. H. 1966. Insulin: Inducer of glucose-6-phosphate dehydrogenase. *Life Sci.* 5:1139-1146.
- Weber, G. and Lea, M. A. 1967. The molecular correlation concept. In *Methods in Cancer Research*, ed. H. Busch, vol. 2, pp. 523-578. New York: Academic.
- Weber, G. and Morris, H. P. 1963. Comparative biochemistry of hepatomas. III. Carbohydrate enzymes in liver tumors of different growth rates. *Cancer Res.* 23:987-994.
- Wharton, D. C. and Tzagoloff, A. 1967. Cytochrome oxidase from beef heart mitochondria. In *Methods in Enzymology*, eds. R. W. Estabrook and M. E. Pullman, vol. 10, pp. 245-250. New York: Academic.
- Winberry, L. and Holten, D. 1977. Rat liver glucose-6-phosphate dehydrogenase. Dietary regulation of the rate of synthesis. *J. Biol. Chem.* 252:7796-7801.

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