Biochemical Pharmacology, Vol. 36, No. 5, pp. 711–716, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

DECREASED ERYTHROCYTE δ-AMINOLEVULINATE DEHYDRATASE ACTIVITY AFTER STYRENE EXPOSURE

HIROYOSHI FUJITA,*† AKIO KOIZUMI,*‡ TEIJI FURUSAWA§ and MASAYUKI IKEDA*

*Department of Environmental Health, Tohoku University School of Medicine, Sendai 980, Japan; and §Kyoto Industrial Health Association, Kyoto 604, Japan

(*Received* 24 *February* 1986; *accepted* 30 *July* 1986)

Abstract—δ-Aminolevulinate dehydratase (ALA-D:porphobilinogen synthase, 5-aminolevulinate hydro-lyase, EC 4.2.1.24) activity was depressed markedly in red cells of rats exposed to 0.21 g/m^3 styrene, a chemical widely used in commercial products. The depression was not restored *in vitro* after treatment with dithiothreitol and zinc. Consistent with this finding, radioimmunoassay of the enzyme protein demonstrated reduction in the enzyme concentration by styrene exposure. There was a good correlation between the decrease in enzyme activity and its concentration in the styrene-treated animals, suggesting that the depression of the enzyme activity was essentially due to the reduction in the enzyme content. Decrease in the enzyme content in bone marrow cells to almost the same extent as that in erythrocytes seems to indicate the decreased synthesis of ALA-D in the bone marrow. *In vitro* studies showed that styrene 7,8-oxide, the major intermediate of styrene metabolism, decreased the activity of purified ALA-D but that styrene, the parent compound itself, had no inhibitory effect. The activity and concentration of erythrocyte ALA-D in workers chronically exposed to styrene were also depressed significantly. These findings indicate that the styrene exposure-mediated decrease of ALA-D activity in erythrocytes was a reflection of reduction in the enzyme protein, which may have been the result of styrene 7,8-oxide action, and they suggest that a similar process may also be involved in the reduction of erythrocyte ALA-D in styrene-exposed workers.

δ-Aminolevulinate dehydratase (ALA-D:porphobilinogen synthase, 5-aminolevulinate hydro-lyase, EC 4.2.1.24) catalyzes the conversion of two molecules of δ-aminolevulinic acid to porphobilinogen, the immediate precursor to porphyrin synthesis [1]. It is well known that ALA-D activity is critically dependent on essential SH groups of the enzyme [2], and that the enzyme activity is decreased markedly by agents which modify SH, such as lead [2]. Thus, enzyme inhibition is widely accepted as one of the most sensitive signs of lead exposure [3, 4].

Recently, we found that depression of ALA-D activity occurs in rats treated with trichloroethylene [5, 6] or bromobenzene [7]. The decreased enzyme activity seemed to be the result of interaction between the essential SH groups of the enzyme and intermediate(s), which are formed by the mixed-function oxidase system from the parent compound [6, 7]. Styrene, one of the most common materials used in the production of polymers, copolymers, and reinforced plastics, is known to be metabolized mainly by the cytochrome P-450-dependent monooxygenase system, forming styrene 7,8-oxide as a major reactive intermediate [8, 9]. This fact suggests the possibility that styrene exposure also decreases ALA-D activity in a manner similar to trichloroethylene exposure [6] and bromobenzene exposure [7], and that enzyme inhibition may be one of the most sensitive indices of styrene exposure. ALA-D is thought to be one of the rate-limiting steps of heme biosynthesis under some circumstances [6, 10]; however, little attention has been paid to the effects of the solvent on the heme biosynthetic pathway [8, 9]. Production of styrene in the world was as much as 7 million tons in 1977, and it is estimated to reach 12.6 million tons by 1990 [9]. Due to the increase in production, the risk of styrene exposure has increased dramatically not only in industries but also in the general population, through motor vehicle exhaust, tobacco smoke, and ingestion of food products packed in polystyrene containers [9]. Studies on the effect of styrene on the activity of ALA-D in human and animal subjects exposed to the chemical thus appeared important to us.

The present study was undertaken to evaluate the effect of styrene on ALA-D activity in animals and in workers who have been exposed to this chemical. We report in this paper that styrene exposure causes a decrease in erythroid ALA-D activity both in human and animals, and that the decreased enzyme activity can be accounted for by a decrease in enzyme concentration.

METHODS

Materials. ALA-D was purified from rat and human erythrocytes and labeled with [^{125}I] (Amersham International Ltd., Buckinghamshire, U.K.) as described previously [11, 12]. The purified enzyme preparations from both sources were homogeneous in analytical polyacrylamide gel electrophoresis, in

† Present address: The Rockefeller University, 1230 York Ave., New York, NY 10021. Author to whom all correspondence should be sent.

‡ Present address: Department of Entomology, College of Natural and Agricultural Sciences, University of California, Riverside, CA 92521.

both the absence and the presence of sodium dodecyl sulfate. The enzyme preparations were stored at 4° after precipitation with ammonium sulfate (to 55% saturation) in the presence of zinc and dithiothreitol [2]. On the day of use, the activated enzyme was prepared according to the previous report [2, 11]. The specific radioactivity of the [^{125}I]ALA-D from the rat and human was 6.9 and 12.6 μCi/μg respectively.

Antisera against rat and human erythrocyte ALA-D were produced in rabbits [6] and goats [12] respectively. An IgG fraction was purified from antisera using a column of DEAE Affi-Gel blue (Bio-Rad Laboratories, Richmond, CA), and used at dilutions of 1:15,000 (anti-rat enzyme IgG) and 1:45,000 (anti-human enzyme IgG). At these dilutions, both IgG preparations resulted in about 50% of maximal binding of the respective labeled antigen. Ouchterlony double diffusion analysis showed that the antibodies formed a single precipitin line with purified and partially purified ALA-D as reported previously [13].

A goat anti-rabbit IgG serum and a rabbit anti-goat IgG serum were purchased from Medical and Biochemical Laboratories (Nagoya, Japan), and ALA hydrochloride was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals used were of analytical grade. For the determination of direct inhibitory effects of styrene and styrene 7,8-oxide, these compounds were equilibrated in water in order to prepare aqueous solutions. The concentration of each compound in the aqueous solution was determined by gas chromatography. All glassware used was washed with HNO_3/HCl and rinsed thoroughly with metal-free distilled water. Buffer solutions, chemicals, and water were routinely checked with a flameless atomic absorption spectrophotometer to establish metal-free conditions.

ALA-D assay. ALA-D activity was determined in both the absence and the presence of 10 mM dithiothreitol and 0.1 mM zinc acetate [2, 14]. Unless otherwise stated, the enzyme activity described in this paper showed reactivated activity with dithiothreitol and zinc acetate. One unit of enzyme activity was defined as the activity that catalyzes the formation of 1 μmole porphobilinogen at 37° per hr.

To examine the enzyme inhibition *in vitro*, the activated enzyme was incubated with styrene or styrene 7,8-oxide in 100 mM Tris/acetate buffer, pH 7.1, under N_2 at 37°. After incubation, the enzyme activity was determined in the presence of dithiothreitol (10 mM) and zinc acetate (0.1 mM).

The radioimmunoassay of erythroid ALA-D was performed as described previously [11, 12]. Since no immunochemical and enzymological differences including amino acid composition were found between the purified liver and erythroid ALA-D of the rat [13, 15, 16], the same radioimmunoassay was used to determine the amount of hepatic ALA-D, using rabbit anti-rat erythrocyte ALA-D IgG.

Determination of SH groups in ALA-D. To investigate the effect of styrene 7,8-oxide on the enzyme SH groups, activated ALA-D was incubated with 3200 times molar excess styrene 7,8-oxide under N_2 at 4° for overnight. After the incubation the enzyme was reactivated with dithiothreitol and zinc at 37° for 30 min. The mixture was passed through a Sephadex G-50 column (0.75 × 12.0 cm) under N_2 to remove excess zinc and dithiothreitol, and the enzyme fraction was collected to determine the SH groups [2] and activity. The SH groups were classified into three types according to the definition of Tsukamoto *et al.* [2].

Animals exposed to styrene. Twenty male Wistar rats, 18 weeks old and weighing 315 ± 26 g (mean ± SD), were acclimated to 12 hr light/12 hr dark cycles for 1 week prior to the experiment. Food and water were fed *ad lib.* For the exposure to styrene, a servomechanism with four chambers [17] was employed. Styrene concentrations in the chamber air were monitored every 18.5 min by gas chromatography and controlled automatically to maintain the desired concentration of the vapor. The coefficient of variation of the styrene concentration was less than 6% throughout the experiment.

Rats were divided into four groups (five animals per group) as follows: (1) sham-exposed (control); (2) those exposed to styrene vapor at 0.21 g/m^3; (3) those exposed to styrene vapor at 0.43 g/m^3; and (4) those exposed to styrene vapor at 0.85 g/m^3. After 168 hr of exposure, body weight was recorded, and blood was collected from the abdominal aorta to determine hematocrit, lead concentration, ALA-D activity and its concentration. The liver was perfused with isotonic KCl via the abdominal aorta, and wet weight was determined. Bone marrow cells were collected from tibia and femora. Protein concentration and the activity and the concentration of ALA-D were determined in liver and bone marrow cells. The lowest exposure concentration of styrene (0.21 g/m^3) was set according to the current occupational limit of the chemical in Japan (< 0.21 g/m^3), and duration of the exposure was fixed to 7 days since the maturation of erythrocytes in the bone marrow from proerythroblasts usually takes 4–7 days in mammals [18, 19].

ALA-D in styrene-exposed workers. Heparinized blood samples were obtained from twenty-four healthy male subjects (as controls), and nine male workers who had been exposed to styrene at a fiber-reinforced-plastic boat plant. In this type of operation, the exposure level of styrene is known to be sometimes higher than 0.85 g/m^3 [20]. The work history of the subjects studied at the plant ranged from less than 0.5 to 22.5 years (10.3 ± 7.2 years; mean ± SD). Answers to a questionnaire showed that their work-hours under styrene vapor were 36.4 ± 10.4 hr/week (mean ± SD), and that no worker had a history of trichloroethylene exposure. Since the answers also showed that every worker handled all processes of styrene operations (they help each other whenever necessary), these workers were divided into two groups according to work-hours and experience with styrene as follows: those in group A worked 48 hr/week under styrene vapor with more than 8 years history of styrene exposure (N = 4); those in group B were others (N = 5). No significant abnormalities were observed in conventional indices of their health examination. Blood samples were assayed for hematocrit, lead concentration, and ALA-D activity and its concentration.

Table 1. Effects of styrene exposure on δ-aminolevulinate dehydratase activity

Styrene vapor concentration (g/m^3)	Treatment with dithiothreitol and zinc	δ-Aminolevulinate dehydratase activity: Erythrocyte ($\times 10^{-3}$ units/ml packed cell) +	Erythrocyte −	Bone marrow ($\times 10^{-3}$ units/mg protein) +	Bone marrow −	Liver ($\times 10^{-3}$ units/mg protein) +	Liver −
0		226 ± 19	155 ± 22	16.5 ± 1.9	10.3 ± 1.8	7.1 ± 0.5	7.6 ± 1.7
0.21		104 ± 12*	76 ± 14*	10.7 ± 0.7*	6.2 ± 1.9*	7.4 ± 0.7	8.2 ± 0.8
0.43		101 ± 16*	63 ± 28*	11.1 ± 0.4*	7.0 ± 0.5*	6.7 ± 1.4	6.2 ± 1.8
0.85		88 ± 16*	60 ± 11*	10.3 ± 1.4*	6.8 ± 2.3†	7.8 ± 1.5	7.8 ± 1.3

Wistar rats were exposed to styrene for 168 hr. After the exposure was terminated, δ-aminolevulinate dehydratase activities in erythrocytes, bone marrow cells, and the liver of the animals were estimated in the presence or absence of dithiothreitol and zinc. Values are mean ± SD (N = 5).

*, † Significantly different from control at * $P < 0.01$ or † $P < 0.05$.

RESULTS

Depression of erythroid ALA-D activity in styrene-exposed rats. No significant changes in body weight were observed even at the highest dose, i.e. 0.85 g/m^3, but liver weight decreased to 85 ± 7% of the control in this group ($P < 0.05$). Styrene exposure of rats resulted in depression of ALA-D in bone marrow and in erythrocytes, whereas there was no apparent change in hepatic enzyme activity (Table 1). The erythroid enzyme activity was depressed even at the lowest dose, i.e. 0.21 g/m^3 (to about 50 and 60% of the control, in erythrocytes and in bone marrow cells, respectively); however, the depression was not significantly greater when the exposure level increased to 0.85 g/m^3 (Table 1). Results in Table 1 also show that the decreased enzyme activity with styrene exposure essentially could not be reactivated by the addition of zinc and dithiothreitol to the enzyme assay mixture *in vitro*. No abnormal lead concentration was observed in any blood specimen. This fact excludes the possibility that the enzyme activity decrease was attributable to lead.

Decrease in the ALA-D content by styrene exposure. To clarify whether the depression of ALA-D activity was due to enzyme inhibition or to enzyme protein decrease, radioimmunoassay was carried out to determine the enzyme concentration.

Table 2 demonstrates the changes in erythroid ALA-D concentration in rats treated with styrene. The enzyme concentration in erythrocytes and bone marrow cells decreased to about 50 and 60% of the control, respectively, in the 0.21 g/m^3 styrene-exposed rats, while the enzyme concentration in the liver did not show any decline. Both the activity and the concentration of ALA-D in erythrocytes decreased proportionally with a correlation coefficient of 0.93 ($N = 20$, $P < 0.01$). A good correlation was also observed between erythrocyte and bone marrow cell ALA-D concentrations ($r = 0.91$, $N = 20$, $P < 0.01$).

Decreases in erythrocyte ALA-D activity and its protein in workers chronically exposed to styrene. The activity and the amount of erythrocyte ALA-D were also determined in nine subjects who had been working at a fiber-reinforced-plastic boat plant as well as in eighteen healthy normal subjects (Table 3). As fluctuations in styrene concentration in the workroom are reported to be very common in this type of plant [9] and answers to a questionnaire showed that workers had few special processes in styrene operation, they were divided into groups A and B according to their work experience and work-hours with styrene. Workers in groups A and B worked 48 hr/week and 31.6 ± 13.0 hr/week (mean

Table 2. Effects of styrene exposure on δ-aminolevulinate dehydratase concentration

Styrene vapor concentration (g/m^3)	δ-Aminolevulinate dehydratase concentration: Erythrocytes (μg/ml packed cell)	Bone marrow (μg/mg protein)	Liver (μg/mg protein)
0	8.8 ± 1.0	0.92 ± 0.14	0.60 ± 0.10
0.21	4.4 ± 1.4*	0.56 ± 0.10*	0.70 ± 0.11
0.43	4.2 ± 1.2*	0.65 ± 0.04†	0.62 ± 0.17
0.85	4.1 ± 0.7*	0.61 ± 0.04†	0.78 ± 0.23

Wistar rats were exposed for 168 hr, and δ-aminolevulinate dehydratase concentrations in erythrocytes, the bone marrow, and the liver were estimated by radioimmunoassay. Each value is the mean ± SD (N = 5).

*, † Significantly different from control at * $P < 0.01$ or † $P < 0.05$.

Table 3. Activity and concentration of δ-aminolevulinate dehydratase in styrene-exposed workers

Workers	δ-aminolevulinate dehydratase activity (units/ml packed cells)	δ-Aminolevulinate dehydratase concentration (μg/ml packed cells)	Activity/concentration ratio of δ-aminolevulinate dehydratase (units/μg enzyme)
Control	2.00 ± 0.30	165 ± 56.8	15.8 ± 6.73
Styrene-exposed			
Group A	1.33 ± 0.45*	83.7 ± 16.4†	17.1 ± 5.41
Group B	1.87 ± 0.28	119 ± 49.9	17.9 ± 6.96

Erythrocyte δ-aminolevulinate dehydratase concentration and reactivated enzyme activity were determined in blood specimens from styrene-exposed workers (N = 9) and healthy controls (N = 18). Workers were divided into two groups according to work-hours and work experience under styrene vapor as follows: (group A) those who worked 48 hr/week under styrene vapor with more than 8 years history of styrene exposure; (group B) others, i.e. those who worked less than 48 hr/week under styrene vapor or who had less than 8 years history of styrene exposure. Each value is the mean ± SD.

*, † Significantly different from control at * $P < 0.02$ or † $P < 0.01$.

± SD), respectively, under styrene vapor, and had 13.4 ± 5.8 years and 6.3 ± 5.1 years (mean ± SD), respectively, of work experience with styrene. Significant decreases were found both in the enzyme activity (to 66 ± 22% of the control, $P < 0.02$) and in the enzyme concentration (to 51 ± 10% of the control, $P < 0.01$) in group A, and both values in group B were between those in the control group and group A (Table 3). This observation suggests an effect of prolonged styrene exposure on erythroid cells. The activity/enzyme protein ratios were similar among these three groups (Table 3), suggesting that the decrease in the enzyme activity in the styrene-exposed subjects was due to a reduction in the enzyme concentration. Blood lead concentrations of the control group, group A, and group B were 6.2 ± 2.2, 5.5 ± 3.0 and 6.5 ± 2.7 μg/100 ml respectively.

In vitro *effects of styrene and styrene 7,8-oxide on ALA-D activity*. The major route of styrene metabolism is via styrene 7,8-oxide [8, 9]. *In vitro* addition of styrene (0.58 to 2.61 mM), i.e. up to 195,000 times molar excess of the concentration of ALA-D, had no effect on the enzyme activity (data not shown). In contrast, enzyme activity decreased dose dependently *in vitro* with the addition of styrene 7,8-oxide. The activity of ALA-D (0.0613 μM) decreased to 66 and 38% of control after incubation with 0.9 and 1.8 mM styrene 7,8-oxide, respectively, and was almost nil with 7 mM styrene 7,8-oxide, i.e. 110,000 times the enzyme concentration. Incubation with 3.7 mM styrene 7,8-oxide decreased the activity of ALA-D (0.0613 μM) in a time-course dependent manner, and only 10% of the activity was detectable after 90 min of incubation. *In vitro* inhibition of ALA-D by styrene 7,8-oxide was not reactivated by the addition of dithiothreitol and zinc.

An SH titration experiment showed that only type I SH groups, which were assumed to be the essential SH groups [2], decreased significantly (from 2.2 moles/subunit to 1.4 moles/subunit) when ALA-D was incubated with 3200 times molar excess styrene 7,8-oxide. The enzyme activity also decreased to 67% of the control.

DISCUSSION

The present study demonstrates depression of erythroid ALA-D activity which could not be reactivated by dithiothreitol and zinc treatment, in animals (Table 1) and, for the first time, in humans (Table 3) exposed to styrene. Our data in rats exposed to styrene vapor show that the depression of enzyme activity was proportional to the reduction in the concentration of the enzyme protein (Fig. 1) even at the 0.21 g/m^3 styrene exposure (Tables 1 and 2). Previous studies show that toxicities of styrene, such as minor changes in axonal proteins [21], enhanced activities of both the drug-hydroxylating (ethoxycoumarin *O*-deethylase, cytochrome P-450) and -conjugating (epoxide hydrolase, UDP glucuronyltransferase) enzymes in the liver and kidneys

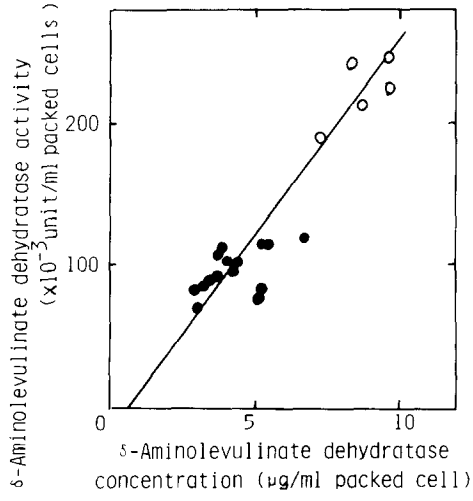

Fig. 1. Correlation between the activity and the enzyme concentration of ALA-D. Determinations were carried out on blood specimens from control (○) (N = 5) and styrene-exposed (●) (N = 15) rats. A regression for least-squares best-fit analysis was $Y = 28X - 19$ (N = 20, $r = 0.93$), where X is the enzyme concentration and Y is the enzyme activity.

[22], liver hydropic degeneration and steatosis of parenchymal cells and congestion, and significant reduction in GSH concentration [23], are demonstrable in rats when the exposure level of styrene exceeds 1.26 g/m^3 [9]. ALA-D decrease in rat erythroid tissues seems to be one of the most sensitive signs of styrene exposure. Similar depressions of enzyme activity and in enzyme concentration were also found in erythrocytes of human subjects occupationally exposed to styrene for extended periods of time (Table 3). The enzyme decrease in human erythrocytes seems to correlate with work-hours and work experience under styrene vapor (Table 3). A similar phenomenon was observed between work-hours of lead-exposure workers and urinary excretion of δ-aminolevulinic acid [24], one of the signs of lead exposure. Present observation seems to suggest a dose–response effect of styrene on human erythrocyte ALA-D. In contrast to the marked decrease in the erythroid enzyme, hepatic ALA-D was not affected (Tables 1 and 2).

Decreases in erythrocyte ALA-D activity have been shown to occur in lead poisoning [25–27], in trichloroethylene exposure [5, 6], in patients with hereditary tyrosinemia [28, 29], and in a rare form of homozygous deficiency of this enzyme [30]. Lead is known to inhibit the enzyme activity by interacting with the essential SH groups in the enzyme protein [2], whereas trichloroethylene seems to inhibit the enzyme activity via formation of a reactive inhibitory intermediate(s) for ALA-D [6]. Patients with hereditary tyrosinemia are known to produce succinylacetone, a potent competitive inhibitor of ALA-D, and the enzyme activity is inhibited in the liver and in erythroid tissues by succinylacetone without abnormality in the enzyme synthesis [31, 32]. The decrease in enzyme activity in the homozygous ALA-D deficiency was associated with disproportionally elevated levels of the immunoreactive enzyme protein for the decreased enzyme activity, suggesting that these patients have a cross-reactive material positive mutation [30]. In contrast to these disorders, rats and human subjects exposed to styrene were found to have proportional decreases of enzyme activity and its concentration (Fig. 1 and Table 3). In other words, the depression of enzyme activity due to styrene exposure was not the result of inhibition of the catalytic activity but of a reduction in the enzyme protein.

It should be noted that ALA-D activities in erythrocytes and bone marrow cells were decreased to a similar level independently of styrene vapor concentration in the air (Tables 1 and 2). One possible reason for this finding is that a metabolite(s) of styrene, rather than the parent compound, decreased the enzyme and that the amount of the metabolite(s) formed was determined by a rate-limiting step in the styrene metabolic pathway. The fact that the enhanced activities of drug-hydroxylating enzymes in the liver were observed when rats were exposed to 1.26 g/m^3 styrene may support this hypothesis, since the present exposure level was below 0.85 g/m^3.

Styrene is known to be metabolized mainly to styrene 7,8-oxide [8, 9]. Styrene 7,8-oxide is highly reactive and can bind covalently to nucleic acids and proteins [33, 34]. It is possible, therefore, that this intermediate may also react with ALA-D, an SH-dependent enzyme. Evidence to support this hypothesis is the finding that the addition of styrene 7,8-oxide depressed the enzyme activity in a dose- and time-dependent manner, though the addition of styrene itself failed to inhibit the enzyme activity. Proportional decreases in the enzyme activity (to 67% of the control) and in the type I SH groups (to 63% of the control) suggest that the enzyme inhibition was caused by the binding between styrene 7,8-oxide and the essential SH groups, since the type I SH groups were assumed to be the essential SH groups by Tsukamoto *et al.* [2]. Decreases in the enzyme activity and titratable SH groups by styrene 7,8-oxide *in vitro* could not be prevented or restored by dithiothreitol and zinc. This finding is in contrast to those observed with trichloroethylene, as the trichloroethylene-mediated inhibition is prevented by dithiothreitol [6]. Alternatively, upon reaction with styrene 7,8-oxide, a structurally altered enzyme protein may be formed, which has not only a decreased activity but also an altered reaction with specific antibody against the normal enzyme. In fact, overnight incubation of 0.9 μM ALA-D with 17 mM styrene 7,8-oxide decreased the enzyme protein which could be detected by radioimmunoassay [16]. Such an altered enzyme protein may also degrade faster *in vivo* than unmodified enzyme, resulting in a proportional decrease in the enzyme activity and in the enzyme protein. Lastly, styrene 7,8-oxide may interfere with the synthesis of ALA-D in erythroid precursors in the bone marrow. Our data also show that both the activity and the concentration of ALA-D in bone marrow cells decreased in rats exposed to styrene. Moreover, there was a good correlation between the bone marrow and erythrocyte ALA-D concentrations, suggesting a decrease in ALA-D synthesis in the bone marrow. Unlike trichloroethylene exposure, however, decreases in ALA-D by styrene were observed only in erythrocytes and bone marrow cells and not in the liver (Tables 1 and 2). This finding suggests that liver detoxifies the reactive intermediate(s) of styrene more rapidly than the erythroid tissues do, since no evidence suggests that the enzymes in the liver and erythroid tissues are isozymes [13, 16, 35]. Inhibition of ALA-D in erythroid tissues of animals exposed to lead is greater than that in the liver [15, 36], and this phenomenon is at least partially attributable to a higher GSH level in the liver than in the bone marrow [15]. A depression in hepatic ALA-D activity in rats treated with bromobenzene is preceded by a decrease in liver GSH concentration [7]. A portion of styrene 7,8-oxide is conjugated with GSH in the liver [37–39], and significant reduction of the liver GSH has been observed in rats exposed to styrene not at 0.42 g/m^3 [22] but at 1.26 g/m^3 [23]. It is suggested, therefore, that rat liver GSH in the present study was not reduced significantly, and that the liver GSH may detoxify the reactive intermediate(s) of styrene. Thus, although both trichloroethylene and styrene produce reactive intermediates via the cytochrome P-450-dependent mixed-function oxidase system, the mechanisms leading to the depression of ALA-D activity by these toxic chemicals are quite different.

Acknowledgements—The authors are grateful to Dr. S. Sassa (The Rockefeller University, New York) for his interest in, and helpful discussions on, this work. We also express our gratitude to Mr. K. Sato, Mr. K. Kurasako, Mr. T. Maruhata (Kyoto Industrial Association, Kyoto), and Mr. and Mrs. Ohta (Radioisotopic Research Center, Tohoku University School of Medicine, Sendai) for their technical assistance in this work.

REFERENCES

1. D. Shemin, in *The Enzymes* (Ed. P. D. Boyer), 3rd Edn, Vol. 7, p. 323. Academic Press, New York (1972).
2. I. Tsukamoto, T. Yoshinaga and S. Sano, *Biochim. biophys. Acta* **570**, 167 (1979).
3. *Biologic Effects on Atmospheric Pollutants, Lead,* p. 71. National Research Council, Committee on Biologic Effects of Atmospheric Pollutants, National Academy of Science, Washington, DC (1972).
4. S. Sassa, in *Heme and Hemoproteins* (Eds. F. de Matteis and W. M. Aldridge), p. 333. Springer, Berlin (1978).
5. A. Koizumi, H. Fujita, T. Sadamoto, M. Yamamoto, M. Kumai and M. Ikeda, *Toxicology* **30**, 93 (1984).
6. H. Fujita, A. Koizumi, M. Yamamoto, M. Kumai, T. Sadamoto and M. Ikeda, *Biochim. biophys. Acta* **800**, 1 (1984).
7. A. Koizumi, H. Fujita, T. Sadamoto, T. Ohmachi, M. Watanabe and M. Ikeda, *Toxicology* **32**, 1 (1984).
8. E. E. Sandmeyer, in *Patty's Industrial Hygiene and Toxicology* (Eds. G. D. Clayton and F. E. Clayton), 3rd Revised Edn, Vol. 2, p. 3312. John Wiley, New York (1981).
9. *Environmental Health Criteria,* Vol. 26, *Styrene*, p. 11. International Programme on Chemical Safety, World Health Organization, Geneva (1983).
10. H. Bonkowsky, J. F. Healy, P. R. Sinclair, J. F. Sinclair and S. I. Shedlofsky, *J. clin. Invest.* **71**, 1175 (1983).
11. H. Fujita, Y. Orii and S. Sano, *Biochim. biophys. Acta* **678**, 39 (1981).
12. H. Fujita, K. Sato and S. Sano, *Int. Archs occup. environ. Hlth* **50**, 287 (1982).
13. M. Yamamoto, H. Fujita, N. Watanabe, N. Hayashi and G. Kikuchi, *Archs Biochem. Biophys.* **245**, 76 (1986).
14. H. Fujita, K. Sato and M. Ikeda, *Ind. Hlth* **20**, 199 (1982).
15. H. Fujita, R. Yamamoto, K. Sato and M. Ikeda, *Toxic. appl. Pharmac.* **77**, 66 (1985).
16. H. Fujita, A. Koizumi, N. Hayashi and M. Ikeda, *Biochim. biophys. Acta,* **867**, 89 (1986).
17. A. Koizumi and M. Ikeda, *Am. ind. Hyg. Ass. J.* **42**, 417 (1981).
18. O. W. Schalm, N. C. Jain and E. J. Carroll, *Veterinary Hematology*, 3rd Edn, p. 356. Lea & Febiger, Philadelphia PA (1975).
19. P. L. Altman and D. S. Dittmer (Eds.), *Biology Data Book*, 2nd Edn, Vol. 1, p. 100. F.A.S.E.B., Rockville MD (1973).
20. M. Ikeda, A. Koizumi, M. Miyasaka and T. Watanabe, *Int. Archs occup. environ. Hlth* **49**, 325 (1982).
21. H. Savolainen and P. Pfaeffli, *Acta neuropath.* **40**, 237 (1977).
22. H. Vainio, J. Jaervisalo and E. Taskinen, *Toxic. appl. Pharmac.* **49**, 7 (1979).
23. E. Elovaara, H. Vainio, P. Pfaeffli and Y. Collan, *Med. Biol.* **57**, 321 (1979).
24. K. Sato, H. Fujita, S. Inui and M. Ikeda, *Int. Archs occup. environ. Hlth* **52**, 25 (1983).
25. Y. Tanabe, *J. Nation's Hlth* **28**, 552 (1959) [English abstract].
26. K. Koike, *J. Nation's Hlth* **28**, 612 (1959) [English abstract].
27. H. C. Lichtman and F. Feldman, *J. clin. Invest.* **42**, 830 (1963).
28. B. Lindblad, S. Lindstedt and G. Steen, *Proc. natn. Acad. Sci. U.S.A.* **74**, 4641 (1977).
29. S. F. Strife, E. L. Zuriweste, E. A. Emmet, V. N. Finelli, H. G. Petering and H. K. Berry, *J. Pediat.* **90**, 400 (1977).
30. H. de Verneuil, M. Doss, N. Brusco, C. Beaumont and Y. Nordmann, *Hum. Genet.* **69**, 174 (1985).
31. D. F. Tschudy, R. A. Hess and B. C. Frykholm, *J. biol. Chem.* **256**, 9915 (1981).
32. S. Sassa and A. Kappas, *J. clin. Invest.* **71**, 625 (1983).
33. J. Marniemi, E. M. Suolinna, N. Kaatinen and H. Vainio, in *Microsomes and Drug Oxidations* (Eds. V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook and A. H. Conney), p. 698. Pergamon Press, London (1977).
34. J. van Anda, B. R. Smith, J. R. Fouts and J. R. Bend, *J. Pharmac. exp. Ther.* **211**, 207 (1979).
35. C. S. Chang, S. Sassa and D. A. Doyle, *Biochim. biophys. Acta* **797**, 297 (1984).
36. J. A. Millar, V. Battistini, R. L. C. Cumming, F. Carswell and A. Goldberg, *Lancet* **2**, 695 (1970).
37. F. Oesch, *Xenobiotica* **3**, 305 (1973).
38. M. O. James, J. R. Fouts and J. R. Bend, *Biochem. Pharmac.* **25**, 187 (1975).
39. J. Pachecka, P. Gariboldi, L. Cantoni, G. Belvedere, E. Mussini and M. Salmola, *Chem. Biol. Interact.* **27**, 313 (1979).