INTRODUCTION

Arsine (AsH₃) is a gas used in organic synthesis and in the semiconductor industry for epitaxial growth of gallium-arsenide (GaAs) and as a dopant for silicon-based electronic devices (ACGIH, 2007). Exposure to arsine is possible from accidental release of the gas during these manufacturing processes. It can also be accidentally generated from arsenic-containing substances, such as wastes from metal/mining and recycling of GaAs semiconductors (ACGIH, 2007). In one such recycling factory, acute poisoning occurred as described in our previous report (Yoshimura et al., 2011). In that instance, percutaneous absorption was suggested because the victim wore a gas mask.
Of the several hundred cases of arsine exposure recorded, 25% were fatal (Fowler and Weissberg, 1974). The main cause of death in humans is kidney damage due to hemolysis after inhalation. Levvy (Levvy, 1947) speculated that the high toxicity of arsine at higher concentrations may be due to the action of unreacted arsine gas on vital organs. However, in animal experiments, no kidney damage was reported after a single arsine exposure, although spleen damage was reported (Blair et al., 1990).

Although many poisoning cases have been reported, there is no information about whether poisoning can occur through percutaneous absorption (ACGIH, 2007). Furthermore, human metabolites in urine and blood after inhalation of arsine (Apostoli et al., 1997, Yoshimura et al., 2011) are the same as the metabolites found in inorganic arsenic exposure. Thus, the effects of inorganic arsenic toxicity should be considered in chronic arsine exposure.

An in vitro study on the dermal uptake of arsenic compounds, inorganic arsenic, arsenosugars, and their metabolites through human skin has been reported (Ouypornkochagorn and Feldmann, 2010). The uptakes of these substances from skin depend strongly on their chemical forms (Ouypornkochagorn and Feldmann, 2010). Arsine has high hydrophobicity in spite of its instability in oxygen, so we presumed that it would be absorbed efficiently through the skin. However, there are no useful data about the transdermal absorbency of arsine.

An in vitro experiment indicated the possible formation of an unidentified arsenic-adduct from arsine-exposed human blood samples (Higashikawa et al., 2008). Although no data were available whether such an arsenic-adduct could be produced in vivo, the formation of such adducts could be a good indicator of arsine poisoning and also lead to elucidation of the induction mechanism of hemolysis by arsine, its main mode of toxicity. Thus, it is necessary to measure the specific arsine metabolites such as arsenic–hemoglobin (As-Hb) adduct in the blood.

Therefore, in order to determine whether arsine can be absorbed percutaneously, we performed acute inhalation studies at the level of almost human lethal dose of arsine gas (ACGIH, 2007) consisting of whole-body inhalation and transdermal exposure using hairless mice, whose skin is in many ways similar to that of humans and is more permeable than human skin (Hinz et al., 1989). In addition, blood samples obtained from arsine-exposed hairless mice were analyzed using high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and spectrophotometry to verify the production of arsenic-adduct.
3, and 6 hr after the end of a single exposure and assessed it by using Ht capillary tubes.

**Total arsenic concentration in whole blood**

Each mouse was killed by exposure to isoflurane 6 hr after the end of a single exposure to arsine; the blood sample was obtained transcardially by using a syringe. Half of the blood sample taken was stored as whole blood at -80°C until analysis.

For total arsenic measurement, 100 μl of whole blood was diluted 10-fold with 0.1 M nitric acid (TAMAPURE AA-100, Tama Chemicals, Kawasaki, Japan), depro-
teinized with an Amicon Ultra filter (Ultracel 100 kDa, Millipore, Billerica, MA, USA), and analyzed using an ELAN DRC-II ICP-MS (PerkinElmer SCIEX, Concord, Ontario, Canada). The instrument settings of the ICP-MS were as follows: RF power 1300 W, argon plasma gas flow 15 l/min, auxiliary flow 1.2 l/min, and nebulizer flow 1.0 l/min. A coaxial-type nebulizer was used; skimmer and sample cones were platinum. ICP-MS detection mass was set as m/z 75 (^{75}As^+), m/z 77 (^{40}Ar^{37}Cl^+), m/z 82 (^{82}Se^+), and m/z 72 (^{76}Ge^+). {^{75}Ge}^+ was used as an internal standard. According to the EPA Standard Methods (EPA, 2009), signal intensity (counts per second, CPS) of arsenic in blood samples was corrected by the following equation in order to remove interference by chlorine in the sample; arsenic CPS = 75CPS – 3.127 × [77CPS – (0.815 × 82CPS)]. The accuracy of the analysis was assessed using Seronorm Trace Elements Whole Blood L-3 (Lot No: 0512627, Sero AS, Billingstad, Norway) as a reference material.

**Detection of As-Hb adduct**

Half of the blood obtained from heart puncture was centrifuged at 2,000 rpm for 10 min to separate the plasma and blood cells and was then placed in a sealed plastic tube and stored at -80°C until analysis. In order to detect As-Hb adduct, we used the blood cells sample. Since the blood cells obtained from mice with whole-body exposure were hemolytic, the supernatant after centrifugation at 2,000 rpm for 10 min was used for the As-Hb adduct analysis. The blood cells from control mice and mice with transdermal exposure were added to water and centrifuged at 2,000 rpm for 10 min to obtain hemoglobin (Hb) solution. We performed spectrophotometric analysis at 540 nm (a Q band absorption that is characteristic of Hb) in addition to speciation analysis of arsenic compounds by HPLC-ICP-MS. The proteins (including Hb) in the supernatant after separation using an Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA, USA) by using a gel filtration column (Superdex™ 75 10/300GL, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) under the following conditions: mobile phase, 10 mM CH3COONH4 with 0.15 M NaCl at pH 7.2; flow rate, 0.5 ml/min; column temperature, 25°C; and injection volume, 100 μl. For Hb detection, absorbance at 540 nm was measured using a visible wavelength detector, and arsenic and iron were measured using an Agilent 7500a ICP-MS (Agilent Technologies). The instrument conditions for ICP-MS were as follows: RF power, 1400 W; plasma argon gas flow rate, 15 l/min; carrier argon gas flow rate, 0.9 l/min; make-up argon gas flow rate, 0.2 l/min. A coaxial-type nebulizer, copper sampling cone, and nickel skimmer cone were used. ICP-MS detection mass was set as m/z 75 (^{75}As^+), m/z 57 (^{40}Fe^+), m/z 35 (^{35}Cl^+), and m/z 72 (^{76}Ge^+).

**Histopathological examination**

Livers, spleens, kidneys, and lungs of mice were fixed in 10% neutral-buffered formalin and then paraffin-embedded. Paraffin sections were stained using hematoxylin and eosin (HE) for histological examination. Kidney sections were also stained with Berlin Blue for hemosiderin. For immunohistochemical examination, the lung, liver, kidney, and spleen sections were examined with rabbit anti-mouse Hb antibody (MP Biomedicals Cappel, Solon, OH, USA). Autoclave pretreatment was performed before reactions with primary antibodies. Endogenous peroxidase was inactivated using 1% H2O2, and non-specific proteins were blocked with skim milk. Sections were then incubated with the primary antibody overnight at 4°C at a dilution of 1/100. Immunolocalization was performed using the Histofine® Simple Stain (mouse MAX-PO [Rabbit]; Nichirei Biosciences, Inc., Tokyo, Japan) with 3,3′-diaminobenzidine as the chromogen, and the sample was counterstained with hematoxylin.

**RESULTS**

**Hematological changes**

Changes in Ht values after a single exposure to arsine are described in Table 1. Ht values from mice that underwent whole-body exposure to ca. 300 ppm arsine significantly decreased to 15.8% and 8.3% after 3 hr and 6 hr, respectively, and all blood was hemolyzed. Six hours after a single transdermal exposure, on the other hand, neither Ht value decrease nor blood hemolysis was observed.

**Total arsenic concentrations in whole blood**

The accuracy of the analytical procedure was tested by analyzing Seronorm Trace Elements Whole Blood L-3, which is certified as a reference for total arsenic. The total arsenic concentration of the reference material was 24.95 ± 0.45 μg/l (n = 5) and the certified value was 25.0 μg/l (95% confidence interval: 23.7-26.3 μg/l). Thus, the arsenic concentration determined by the present ICP-MS method was within the allowable range for certified values.

The total arsenic concentration in the blood 6 hr after the end of the exposure period is presented in Table 1. Blood was not sampled from 2 mice that underwent whole-body inhalation because they were near death at the sampling time. The mean concentration of arsenic in the blood after transdermal exposure was significant.
ly lower than that after whole-body inhalation exposure, similar to control values.

**Detection of As-Hb adduct in blood**

The combination HPLC-ICP-MS chromatogram with absorbance at 540 nm is shown in Fig. 2. The peak absorbance at 540 nm (found between 43 and 75 kDa) showed high abundance of Fe \( (m/z) 57 \), consistent with the 64.5 kDa mass of the Hb tetramer. In addition, the peak between 29 and 43 kDa is considered to be the Hb dimer. Two arsenic peaks were detected in the elution position of Hb shown in the blood after whole-body inhalation (Fig. 2B), but no arsenic peaks were detected after transdermal exposure (Fig. 2C) or for the control (Fig. 2A). The two arsenic peaks in the samples obtained after whole-body inhalation disappeared following filtration with a cut-off level of 10 kDa (data not shown). These results showed that the arsenic coexisted with Hb.

**Autopsy findings**

Hematuria was evident in the bladders of mice exposed to a single whole-body inhalation, but was not observed in the mice with transdermal exposure. All mice in the whole-body inhalation group had dark spleens. No gross lesions were observed in the control mice or in any of the mice exposed percutaneously.

**Histopathological findings**

Histopathological findings from the livers, kidneys, spleens, and lungs of mice exposed to arsine gas by whole-body inhalation and percutaneous-only route are shown in Table 2. Exposure-related histopathological changes were observed in the kidneys, livers, and spleens of the mice exposed to arsine gas by the whole-body route. In the kidneys, marked depositions of eosinophilic globules were noted in the proximal convoluted tubules of all mice in the whole-body inhalation group (Fig. 3A-1). Numerous eosinophilic globules appeared in the cytoplasm of tubular epithelium cells, and the cytoplasm of almost all proximal convoluted tubules was filled with the eosinophilic globules. The eosinophilic globules were stained strongly with eosin and were round in shape. Their size varied from smaller than erythrocytes to moderately bigger than erythrocytes. In addition to the eosinophilic globules, eosinophilic casts were occasionally observed in the lumen of the distal tubules and Henle’s loops in several mice that were exposed by the whole-body route, but glomeruli appeared normal.

The eosinophilic globules were also observed in the livers and spleens. In the livers, the eosinophilic globules appeared in the cytoplasm of Kupffer cells.

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**Table 1. Changes in hematocrit (Ht) value and total arsenic concentration in the blood of hairless mice after single exposure to arsine via whole-body inhalation or percutaneous-only route**

<table>
<thead>
<tr>
<th>Route of exposure</th>
<th>Mouse No.</th>
<th>Body weight (g)</th>
<th>Exposure concentration of arsine (ppm)</th>
<th>Ht value (%) after exposure</th>
<th>Total arsenic concentration in blood (mg/l)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 hr</td>
<td>3 hr</td>
</tr>
<tr>
<td>Whole-body inhalation</td>
<td>1</td>
<td>23</td>
<td>320</td>
<td>53</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td></td>
<td>50</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22</td>
<td></td>
<td>52</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>21</td>
<td></td>
<td>53</td>
<td>16</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>22.0</td>
<td></td>
<td>52.0</td>
<td>15.8</td>
</tr>
<tr>
<td>Percutaneous-only</td>
<td>5</td>
<td>23</td>
<td>320</td>
<td>55</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>23</td>
<td>300</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>23</td>
<td>320</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>23</td>
<td>300</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>23.0</td>
<td></td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>19</td>
<td>0</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>22</td>
<td>0</td>
<td>50</td>
<td>54</td>
</tr>
</tbody>
</table>

ND: Not Determined
*The total arsenic concentration was analyzed in a blood sample taken 6 hr after the end of the exposure period.
The eosinophilic globules were located within the red pulp in the spleens (Fig. 3C-1). The eosinophilic globules observed in the livers and spleens were stained strongly with eosin, similar to those in the kidneys, and were about 2-3 times larger than erythrocytes.

Immunohistochemically, the eosinophilic globules of the kidneys, livers, and spleens showed a positive reaction to the Hb antibody, indicating that Hb existed in the eosinophilic globules (Fig. 4A). The eosinophilic casts in the distal tubules and Henle’s loops were also positive for Hb. However, the eosinophilic globules did not show a positive reaction to Berlin Blue staining, revealing no evidence of hemosiderin formation (data not shown). Necrotic lesions were not observed in the kidneys, livers, or spleens. Intravascular erythrocytic stain affinity was weak, and the numbers of erythrocytes decreased in all organs examined in the whole-body exposure group. In the lungs of the whole-body exposure group, there were no significant changes except for the erythrocyte change in the blood vessels.

Neither exposure-related histopathological changes nor eosinophilic globules were found in the organs of any mice that were exposed percutaneously as well as control mice.

**DISCUSSION**

These findings suggest for the first time that arsine is not absorbed through the skin of mice. These exposure levels are almost equal to human lethal dose which is reported to be from 70 ppm to 300 ppm (ACGIH, 2007). Dutkiewicz (1977) reported that arsenate was absorbed from rat skin and the rate was 1.14-33.1 μg·cm⁻²·hr⁻¹. In an *in vitro* study using human skin, arsenite and dimethylarsinic acid were taken up percutaneously at rates greater than a factor of 29 and 59 higher than that of arsenate (Ouyornkochagorn and Feldmann, 2010). From these results, we speculated that arsine gas could penetrate human skin. Since there was no data whether arsine gas could be absorbed through human skin, we examined the uptake of arsine through human skin by using hairless mouse as a model, because in this model, arsine gas is in direct contact with the skin and permeability of mouse skin is higher than that of human skin (Hinz *et al*., 1989). However, the result from the present study did not show a toxic effect from ca. 300 ppm for a 5 min transdermal exposure to hairless mice, whereas whole-body inhalation caused severe hemolysis even 3 hr after the end of exposure period, as shown in Table 1.

The critical target system for arsine exposure is reported to be the hematopoietic system, and the impairment of renal function is correlated with the degree of intravascular hemolysis and death rate that the subjects experience (ACGIH, 2007; IPCS, 2001). Our animal experiments clearly expressed the severe typical effect on Ht values from acute arsine exposure in mice after whole-body inhalation at ca. 300 ppm for 5 min. We also observed histopathological changes in organs, including the kidneys, spleens, and livers, caused by inhalation of ars-
**Fig. 3.** Histopathological findings. Hematoxylin and eosin (HE) stain. A-1: Kidney of a mouse exposed to arsine gas by whole-body inhalation. Eosinophilic globules in the cytoplasm of proximal convoluted tubules (arrows), and eosinophilic cast in the lumen of the distal tubules (arrowheads). A-2: Kidney of a control mouse. B-1: Liver of a mouse exposed to arsine gas by whole-body inhalation. Eosinophilic globules in the cytoplasm of Kupffer cells (arrows). B-2: Liver of a control mouse. C-1: Spleen of a mouse exposed to arsine gas by whole-body inhalation. Eosinophilic globules in the red pulp (arrows). C-2: Spleen of a control mouse. Bars indicate 50 μm.

**Table 2.** Histopathological findings in hairless mice exposed to arsine gas by whole-body or percutaneous-only route

<table>
<thead>
<tr>
<th>Route of exposure</th>
<th>Whole-body</th>
<th>Percutaneous-only</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals examined</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophilic globules: proximal convoluted tubules</td>
<td>4</td>
<td>(3+)</td>
</tr>
<tr>
<td>Eosinophilic casts: distal tubules</td>
<td>3</td>
<td>(+ ~ 2+)</td>
</tr>
<tr>
<td>Eosinophilic casts: Henle’s loops</td>
<td>2</td>
<td>(+)</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophilic globules: Kupffer cells</td>
<td>4</td>
<td>(+ ~ 2+)</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophilic globules: red pulp</td>
<td>4</td>
<td>(3+)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Each value indicates the number of lesion-bearing mice. The number in parentheses represents a grade of histopathological findings (+: slight; 2+: moderate; 3+: severe). NS: No significant change in all animals.
No effects in either Ht value or histopathology were observed in hairless mice that experienced the same level of exposure percutaneously.

We found arsenic adduct, presumably As-Hb adduct, in the blood of mice that had experienced whole-body inhalation, but no adduct was observed in either transdermal exposure or control mice, as shown in Fig. 2. An in vitro study showed that arsenic adduct was detected in human blood samples after exposure to arsine for 90 min (Higashikawa et al., 2008). Although the study mentioned that the arsenic adduct detected was derived from erythrocytes during hemolysis and the molecular weight was over 10 kDa, it did not identify whether the arsenic adduct resulted from Hb or other components from erythrocytes. In Fig. 2, arsenic peaks detected after whole-body inhalation (B) disappeared following filtration with a cut-off level of 10 kDa (data not shown). These results indicate that arsenic adduct is larger than 10 kDa and is generated by arsine exposure. It is, however, unknown whether this hemolysis is induced through the formation of the adduct. An earlier study reported that arsine has a stronger hemolytic action at 37°C than at 22-23°C (Hatlelid et al., 1995). Hemolysis by in vivo arsine exposure might occur with relatively higher efficiency than hemolysis by in vitro exposure at room temperature. An in vitro reaction between arsine and Hb showed an increase in heme release, which might lead to the hemolytic action by arsine (Rael et al., 2006). The heme release might occur following the formation of As-Hb adduct.

Intravascular hemolysis causes tetrameric Hb released into plasma to change to its dimeric form (Rother et al., 2005a). Accordingly, Hb released into plasma is composed of a mixture of its dimer and tetramer forms in the early stages of hemolysis. Our samples that originated from hemolyzed plasma by arsine also showed 2 Hb peaks, around 60 kDa as the tetramer and 30 kDa as the dimer form (Fig. 2).

Total arsenic concentrations in the whole blood of mice after percutaneous-only exposure were comparable with those of the control mice and significantly lower than those of mice after whole-body inhalation, as shown in Table 1. Because the blood samples were diluted with 0.1 M nitric acid, erythrocytes were decomposed. Therefore, the total arsenic concentration in blood showed arsenic not only in the plasma but also in erythrocytes. When dog blood was exposed to arsine, arsenic incorporated into red blood cells; binding of arsenic to cell membranes accounted for approximately 75% of the total arsenic detected (Hatlelid et al., 1995). Although no report has traced total arsenic levels in animals after arsine exposure, detection of arsenic adduct, presumably As-Hb adduct, even 6 hr after the end of arsine exposure suggests that inhaled arsine may promptly enter red blood cells. Therefore, we believe that total arsenic in blood can increase immediately after exposure. As the molecular weight of tetramer Hb is about 64.5 kDa, our pretreatment by using a deproteinization filter with a cut-off level of 10 kDa removed the adduct (data not shown) and the result obtained was similar to that in a previous in vitro study.

**Fig. 4.** Immunohistochemical staining of the kidney for hemoglobin (Hb). A: Kidney of a mouse exposed to arsine gas by whole-body inhalation. Both the eosinophilic globules in the cytoplasm of proximal convoluted tubules (arrows) and the eosinophilic cast in the lumen of the distal tubules (arrowheads) showed a positive reaction to the Hb antibody. B: Kidney of a control mouse. Normal erythrocytes in the blood vessels are positively stained (arrows). Bars indicate 50 μm.
study (Higashikawa et al., 2008). Therefore, these results also suggest that arsine is incorporated rapidly into the blood cells and in part binds to Hb. Because these phenomena were not observed after a transdermal exposure, we conclude that arsine is not absorbed through the skin.

Severe histopathological effects were observed in the kidneys, livers, and spleens of all mice with whole-body inhalation, but no changes in any organ were found in mice after transdermal exposure, as shown in Table 2. No effects were observed in lungs after either type of exposure. The lesions in the kidneys were numerous eosinophilic globules in the cytoplasm of the epithelium of the proximal convoluted tubules and casts in the tubular lumen (Fig. 3A-1). These globules and casts contained abundant Hb (Fig. 4A), but glomerular changes were not present at this stage (Figs. 3A and 4). As mentioned above, circulating free Hb in plasma readily passes through the renal glomeruli during a severe hemolytic episode (Bunn et al., 1969), and severe hemolysis is known to cause hemoglobinuria and renal dysfunction (Rother et al., 2005b). Intravascular hemolysis releases Hb into plasma, where Hb quickly binds to haptoglobin (Hp), forming a Hp-Hb complex. In persistent intravascular hemolysis, plasma Hp is consumed and free Hb accumulates in the plasma and dissociates from its usual tetrameric form to dimeric Hb. Dimeric Hb is filtered more easily by the glomerulus and incorporated into proximal tubules, leading to accumulation of ferric ions in these cells (Ballarin et al., 2011). However, no hemosiderin was detected in our experiment, so more than 6 hr may be needed for ferric iron to accumulate in the tubules. Muehrcke and Pirani (Muehrcke and Pirani, 1968) performed a clinical pathology study of a patient with arsine-induced anuria, examining 5 serial renal biopsy specimens by light and electron microscopy. Our findings of tubular lesions filled with eosinophilic globules and casts containing Hb are consistent with the first renal biopsy sample from their patient, which was taken 7 days after exposure. However, at that time, the patient was already receiving various treatments. Muehrcke and Pirani (Muehrcke and Pirani, 1968) mentioned that the severity of tubular lesions was apparently responsible for the prolonged anuria, but at that time, the patient’s glomeruli were apparently normal, as were those of our mice. Therefore, our findings are typical of the first phase of renal damage, which may progress to renal dysfunction and result in death.

The deposition of eosinophilic globules was observed in the Kupffer cells of the liver and in the red pulp of the spleen, as well as in the kidney. These features may indicate a clearance of Hb by phagocytic cells. The Hp–Hb complex binds to CD163 on the surface of macrophages and monocytes, in order to facilitate endocytosis and degradation (Rother et al., 2005a).

Levvy (1947) reported that the relationship between arsine concentration (C) and duration of exposure for 50% death (T) approximates to \( C^2T = \text{constant} \). Because the impairment of renal function is correlated with the degree of intravascular hemolysis and the death rate (WHO, 2001), our experiment may serve as a model for severe acute arsine poisoning.

Hughes and Levvy (1947) speculated that arsine had a toxic action on the liver and kidneys, based on an in vivo experiment by using tissue slices. In addition, Ayala-Fierro et al. (2000) confirmed that unreacted arsine was very toxic to an isolated rat kidney. As toxic effects were observed in glomerular and peritubular endothelial cells in their experiments, they speculated that earlier cytotoxicity caused by unreacted arsine itself results in kidney dysfunction, and further damage is caused by the formation of a hemolysate that may contain arsinite. However, we observed high levels of hemolysate in the proximal convoluted tubules but no effects in the glomerular cells, nor was any hemolysate observed in the lesions from the first renal biopsy of the arsine poisoning patient reported by Muehrcke and Pirani (Muehrcke and Pirani, 1968). We speculate that early cytotoxicity appears in the erythrocytes, and then kidney lesions are caused by the hemolysate.

In our experiment, no changes were observed in the lungs, even in mice that experienced severe hemolysis and kidney damage. In addition, a chest radiograph of an acute severely poisoned human patient was normal 3 days after exposure (Uldall et al., 1970). These results suggest that first arsine enters into erythrocytes in blood vessels and then it destroys the cells without injury to the lung tissue.

In conclusion, these results showed arsine-induced hemolysis and deposition of Hb in the kidneys via inhalation, but arsine was hardly absorbed through the skin. As-Hb adducts were detected after in vivo exposure. Thus, As-Hb adducts might serve as a good confirmation indicator for arsine poisoning.

**ACKNOWLEDGMENTS**

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