DMPS-Arsenic Challenge Test. I: Increased Urinary Excretion of Monomethylarsonic Acid in Humans Given Dimercaptopropane Sulfonate[1]

H. VASKEN APOSHIAN, ALEX ARROYO, MARIANO E. CEBRIAN, LUZ MARÍA DEL RAZO, KATHERINE M. HURLBUT, RICHARD C. DART, DIEGO GONZALEZ-RAMIREZ, HELMUT KREPPEL, HERNAN SPEISKY, ALLAN SMITH, MARIA E. GONSEBATT, PATRICIA OSTROSKY-WEGMAN and MARY M. APOSHIAN

Department of Molecular and Cellular Biology, Life Sciences South Building, The University of Arizona, Tucson, Arizona (H.V.A., M.M.A.), Ministerio de Salud, Servicio de Salud Antofagasta, Antofagasta, Chile (A.A.), Seccion de Toxicologia Ambiental, CINVESTAV-IPN, Mexico, D.F. 07000, Mexico (M.E.C., L.M.D.R.), Rocky Mountain Poison and Drug Control Center, Denver, Colorado (K.M.H., R.C.D.), Department of Pharmacology, Centro Investigacion Biomedica del Noreste, IMSS, Monterrey, Mexico (D.G.R.), University of Munich, Walther Straub Institut für Pharmakologie und Toxikologie, Munich, Germany (H.K.), Biochemical Pharmacology Unit, INTA, Universidad de Chile, Santiago, Chile (H.S.), School of Public Health, University of California at Berkeley, Berkeley, California (A.S.) and Instituto de Investigaciones Biomédicas, UNAM, Circuito Escolar Interior Ciudad Universitaria, 04510 Mexico, DF.(M.E.G., P.O.W.)

Accepted for publication March 17, 1997

ABSTRACT

The purpose of the present study was to evaluate in a novel manner the arsenic exposure of humans living in two towns in Northeastern Chile. Residents of one town drink water containing 593 μg As/l. Those in the control town drink water containing 21 μg As/l. Our hypothesis was that the administration of the chelating agent, 2,3-dimercaptopropane-1-sulfonic acid, Na salt (DMPS, DIMAVAL) would increase the urinary excretion of arsenic, alter the urinary profile of arsenic species and thus result in a better indication of the body load of arsenic and a better biomarker for arsenic exposure. The method used to evaluate these subjects was to give them 300 mg DMPS by mouth, after an overnight fast, and collect urine at specified time periods. The urine samples were analyzed for inorganic arsenic, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and total arsenic by hydride generation and atomic absorption spectrophotometry. The results indicated that: 1) During the 2-hr period after DMPS administration, MMA repre-sented 42%, inorganic As, 20 to 22% and DMA, 37 to 38% of the total urinary arsenic. The usual range of the MMA percent-age in human urine has been 10 to 20%. The % MMA increased almost equally for both the arsenic-exposed and control sub-jects. 2) The exposed subjects had a greater urinary excretion of total arsenic, before and after DMPS administration, than the control subjects. 3) Although buccal cells were obtained only from a few subjects, the prevalence of mononucleated buccal cells, an indication of genotoxicity, was 5-fold greater for those who consumed drinking water with the higher arsenic content than among control subjects. Our conclusions are that 1) DMPS has a highly specific effect in humans on MMA metabolism and/or urinary excretion; 2) the human body stores substantial amounts of arsenic; and 3) the urinary arsenic concentration after DMPS administration may be more indicative of the body burden of arsenic because it was greater than that found before DMPS was given.

Epidemiological evidence indicates that ingestion of ar-senic compounds *via* drinking water can result in skin cancer and cancer of internal organs (Chen *et al.*, 1985), whereas exposure *via* inhalation can lead to cancer of the lungs (IARC, 1980). These carcinogenic results of arsenic exposure have been observed only in the human. Animal models have been reported but are, to say the least, questionable. The drinking of water containing high levels of inorganic arsenic by humans has continued to be of concern at the local and international level. For example, arsenic in the drinking water from the deep artesian wells of Southwest Taiwan has been implicated in the etiology of Blackfoot Disease, a vas-cular disease, which can result in spontaneous or surgical amputation of limbs (Tseng, 1977). The etiology of this dis-ease is controversial, even among Taiwanese investigators (Tseng *et al.*, 1996; Chen *et al.*, 1995). Recent reports indicate that the drinking water from an area where Blackfoot Dis-ease is endemic is different from two control areas in its content of insoluble arsenic and not in its soluble arsenic (Chen *et al.*, 1995). There have been other investigations of populations who drink water with high arsenate/arsenite levels in Chile (Sancha *et al.*, 1992), Mexico (Cebrian *et al.*,

ABBREVIATIONS: DMPS, sodium 2,3-dimercapto-1-propane sulfonate; inorgAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; TotAs, total arsenic = inorg As + MMA + DMA; MN, micronuclei.

1983) and most recently in India (Guha Mazumder *et al.*, 1988). In West Bengal, India, more than 800,000 people have been reported to be drinking water containing 190 to 737 μg arsenic per liter with 175,000 of them showing open lesions related to arsenic exposure (Guha Mazumder *et al.*, 1988; Chatterjee *et al.*, 1995).

In 1994, the Tucson group was asked to study a group of residents of San Pedro de Atacama, Chile who were believed to drink water containing approximately 600 μg As/l. The WHO (1996) recommendation is that arsenic in drinking water should not exceed 10 μg As/l. This population in North-eastern Chile does not have a high incidence of arsenic-related diseases. Neither Blackfoot Disease nor skin cancer has been detected (personal communication, A. Arroyo). Because of our extensive experience with chelating agents (Aposhian, 1983; Aposhian and Aposhian, 1990; Aposhian *et al.*, 1995; Gonzalez-Ramirez *et al.*, 1995; Maiorino *et al.*, 1996), especially with the sodium salt of 2,3-dimercaptopropane-1-sulfonic acid (DMPS, DIMAVAL), it was decided to use this orally active chelating agent to answer four questions. First, will DMPS, an effective mobilizing agent for mercury in humans (Aposhian *et al.*, 1992; Gonzalez-Ramirez *et al.*, 1995; Maiorino *et al.*, 1996; reviewed by Aaseth *et al.*, 1995; Aposhian *et al.*, 1995), mobilize arsenic in humans exposed to inorganic arsenic in their drinking water? Second, is arsenic stored in the human body? Third, will the use of DMPS give a more accurate estimate of the body burden of arsenic in humans? Fourth, do subjects from the exposed town have a body burden of arsenic greater than those from the control town? The results of these studies indicate that the answers to all of these questions are positive. In addition, MMA, which was approximately 14% of the total urinary arsenic before DMPS, increased to 42% during the 2-hr period after DMPS administration. This large MMA percentage in the urine is most unusual for humans.

Materials and Methods

Clinical. Subjects underwent a history and physical examination before enrollment in the study. Female subjects were given a urinary pregnancy test and if positive were disqualified as subjects. The physical examination was repeated 24 hr after DMPS administration. DIMAVAL capsules of the same lot number containing 100 mg DMPS were used in this study and were gifts of Heyl (Berlin, Germany). In Germany, DIMAVAL is registered with the German equivalent of the U.S. Food and Drug Administration. DIMAVAL is the only DMPS preparation that is prepared by acceptable Western World pharmaceutical manufacturing procedures.

Our experimental protocol was approved by the Ethics Committees of the Republic of Chile, Ministry of Health, Health Service Antofagasta, Department of Integrated Attentions to Persons and the University of Chile. Because DIMAVAL is an investigational drug in the United States, the study was performed under the U.S. Food and Drug Administration IND No. 34,682.

Vital brand purified bottle water was used for drinking water during the study. The source was a thermal fountain in Chanqueahue in the Sixth Region of Chile. Before being bottled, the water was purified with porcelain and membrane filters. The most pertinent characteristic of this commercially available water was that the arsenic concentration was less than 0.05 mg/l.

Protocol for the DMPS chelation test. Participants were asked to exclude seafood from their diet for the preceding 3 days. Before DMPS administration, each participant read and signed a consent form, which was written in Spanish. A brief medical and occupa-

tional questionnaire for each subject was filled out by the interviewer after questioning the subject. Inclusion criteria for this study were: adults, 18 to 69 years of age. Exclusion criteria were: subjects with known hypersensitivity to similar chemical chelating agents, subjects with a history of previous chelation therapy, subjects with a history of current physical findings of serious renal or psychiatric disease, subjects with abnormalities in blood tests or urinalysis that in the investigator's opinion would interfere with the evaluation of safety data, subjects who had received any investigational drug during the preceding month before the initiation of this study; subjects who had taken drugs with well-defined organ toxicity within the past 6 months, subjects who were pregnant or lactating, subjects with a history of alcohol or recreational drug abuse and subjects who were not capable of giving informed consent.

Subjects were fasted overnight (minus 11 to 0 hr) before DMPS administration (table 1) during which time they were allowed to drink purified bottled water. Throughout the study, the subjects drank Vital brand purified bottled water. At 0 hr (beginning at 7 A.M.) they were given 300 mg DMPS and encouraged to drink 500 ml purified water. They received no food until 4 hr after DMPS administration, at which time they were given a chicken sandwich and a banana. Urine was collected from 11 hr before the time of DMPS administration and for the following 24 hr according to a predetermined schedule (table 1). The DMPS dose was chosen on the basis of previous studies at The University of Arizona (Maiorino *et al.*, 1991; Aposhian *et al.*, 1992) and other clinical reports (Kemper *et al.*, 1990). This dose was given to each subject independent of the body weight because the protocol was a diagnostic test, not a treatment for toxicity.

Urine collection. All collecting containers had been soaked overnight in 2% nitric acid (Baker analyzed for trace metal analysis) or washed in 20% nitric acid and rinsed with water that had been double distilled and deionized. All plastic measuring and collecting equipment were so washed in Tucson, AZ, sealed in bags, placed in locked foot lockers and transported by air to the site of the study at the same time as the investigators. Urine was collected in a 3-liter polyethylene container (Baxter Laboratories, Inc., Morton Grove, IL); the volume was measured; pH adjusted to 4 to 5 by adding concentrated HCl (Baker analyzed for trace metal analysis); and immediately frozen by placing in a portable icebox containing dry ice. The samples were kept frozen while being transported to Tucson, where they were stored at $-20°C$ for approximately 6 months. At that time, they were thawed by standing at room temperature overnight. The next morning, the samples were mixed by five gentle inversions, 35 ml were transferred to acid-washed plastic containers, quick-frozen and carried frozen by air to Mexico City, where they

TABLE 1

DMPS-arsenic challenge protocol

−11 to 0 hr	Begin fast Begin overnight urine collection
0 hr	End overnight urine collection No breakfast, no coffee, no tea Administer three 100-mg DMPS capsules Begin 0–2 hr urine collection
2 hr	End 0–2 hr urine collection Begin 2–4 hr urine collection
4 hr	End 2–4 hr urine collection Begin 4–6 hr urine collection Chicken sandwich
6 hr	End 4–6 hr urine collection Begin 6–24 hr urine collection
11 hr	Dinner
24 hr	End 6–24 hr urine collection

Physical examinations were given and vital signs were measured before and after the study.

were analyzed in the CINVESTAV Laboratory for arsenic species by hydride generation atomic absorption spectrophotometry. The analyst did not know the identity of the samples or the village from which they were obtained.

Total arsenic analysis. An aliquot of urine (0.5–3.0 ml) was wet digested with nitric, sulfuric and perchloric acids according to Cox (1980). All digested samples were pretreated at room temperature with 0.5 ml of 10% (w/v) potassium iodide solution and 2.5% (w/v) ascorbic acid solution for 0.5 hr before measurement. Digested urine samples were analyzed in a Perkin Elmer 3100 atomic absorption spectrophotometer equipped with a flow injection atomic spectroscopy system (FIAS-200). All measurements were made with an arsenic electrodeless discharge lamp. The detection limit of this technique for total arsenic is 5 $\mu g/l$.

The reagents for flow-injection analysis and hydride generation were sodium borohydride 0.2% in 0.05% sodium hydroxide used as a reductant and 2% hydrochloric acid used as an acidic carrier solution for the hydride generation. Argon was the carrier gas.

Arsenic species analysis. Aliquots of urine samples were digested with 2 M hydrochloric acid for 5 hr at 80°C. Arsenic species were separated according to Crecelius (1986). Arsenicals were reduced to their corresponding hydrides and then detected with use of a Varian model 475 atomic absorption spectrophotometer. In the procedure, inorgAs, MMA and DMA were selectively reduced to the gaseous compounds arsine, methylarsine and dimethylarsine by controlled pH and with sodium borohydride as a reducing agent. Arsines were then trapped in a liquid nitrogen-cooled chromatographic trap, which upon warming, allowed a separation of arsenic species based on boiling points. The released arsines were swept by helium carrier gas into a quartz cuvette burner cell, where they were decomposed to atomic arsenic. The system was calibrated by the analysis of standards that contained inorgAs (+5), MMA (+5) and DMA (+5). Because standard urine containing known amounts of As species was not available commercially, the reliability of the separation procedures was assessed by spiking urine samples with known amounts of inorgAs, MMA and DMA (20:20:60), so that three different concentrations (45, 420 and 1150 $\mu g/l$) of TotAs could be analyzed (Vahter and Lind, 1986). Recoveries ranged from 92 to 114% with coefficients of variation between 0.5 and 12%. Freeze-dried urine standard reference material for toxic metals (SRM 2670, National Institute of Standards and Technology [NIST], Gaithersburg, MD) was analyzed for TotAs. The certified concentration of the standard was 480 μg As/l. We obtained 511 μg As/l (range, 431–545). Our accuracy was 90 to 113%. In addition, the laboratory participates in the laboratory intercomparison program for As speciation in human urine, which is coordinated by Dr. E. Crecelius, Battelle, Marine Sciences Laboratory, Pacific Northwest Division, Sequim, WA. Our detection limit for arsenic species was 1 ng for inorgAs, 2 ng for MMA and 4 ng for DMA. The results obtained had an accuracy of 90 to 108% and 2 to 13% coefficient of variation.

Analysis of total arsenic in water was carried out as reported previously (Del Razo *et al.*, 1990). For quality control purposes, the Standard Reference Water (1643c, NIST) was analyzed for TotAs (certified concentration 82.1 $\pm$ 1.2 μg As/l) at the same time as the collected water samples. The results obtained had an accuracy of 95 to 105% and 2 to 7% coefficient of variation.

Creatinine in urine. Creatinine was measured by a colorimetric automated method with use of a Vitalab Eclipse, Merck spectrophotometer. Arsenic concentrations in urine were expressed as micrograms per gram of creatinine.

Micronuclei assay. Buccal cells were collected by gently rubbing the inside of the mouth with a premoistened wooden applicator, which was then dipped into isotonic saline solution in a 15-ml plastic centrifuge tube. The cells were allowed to fall into the solution and gently pipetted to reduce cell clumping. The test tubes were kept on ice after they were collected and transported by air in a small camping icebox containing ice to Mexico City, Mexico with one of the investigators. The cells were centrifuged for 10 min at 200 $\times$ *g,* fixed

with methanol for 1 hr and placed on a clean glass slide. Slides were air dried and preparations were Feulgen-stained by pretreatment with 1 N HCl for 5 min at room temperature, placed for 6 min in 1 N HCl at 60°C, rinsed with distilled water, put into Shiff's reagent for 60 to 90 min and rinsed in tap water. The presence of micronucleated cells was confirmed by three different analysts with the criteria described by Tolbert *et al.* (1992). The analysts did not know whether they were examining buccal preparations from San Pedro de Atacama or Toconao.

Results

Site of study. San Pedro de Atacama was our study town. Toconao was the control town. They are relatively isolated in the Atacama Desert in northeast Chile, more specifically in the El Loa Province of the Second Region (Antofagasta Region) of Chile (fig. 1). San Pedro de Atacama is a 5- to 6-hr automobile drive from the city of Antofagasta, is approximately 2437 meters above sea level and is situated on the banks of the Vilama and San Pedro Rivers. The Vilama River has an arsenic concentration of 593 $\mu g/l$, and the San Pedro River, 220 $\mu g/l$. This is the average of monthly analyses performed by the Health Services Laboratory of the Second Region. This is not an anthropogenic pollution. It is believed that the water has contained high levels of arsenic for centuries and that the source of the arsenic in the area is the runoff from high volcanic formations. In addition, the arsenic content of the water has seasonal variations. Toconao is approximately a 1-hr drive beyond San Pedro de Atacama and is 2477 meters above sea level. In Toconao, the people drink water from the Onar-Jerez Creek (19 μg As/l) and the Silapeti River (15 μg As/l).

There is a rudimentary medical dispensary in each of these towns. They are in contact by radio, when necessary, with the Regional Health Department located in the city of Antofagasta. The most common diseases are acute respiratory diseases in the winter and acute enteric diseases in the summer. The medical dispensary of each town was where the physical examinations of each subject were performed, DMPS administered and urine collected and measured. The Secretary of Health for the Second Region is Dr. Alex Arroyo-Meneses, whose medical specialty is dermatology and who is still active in the practice of this specialty.

The local economies of San Pedro de Atacama and Toconao are based, in order of their importance, on agriculture, tourism and craftsmanship. In addition, Toconao has two other industries, lithium mining and extraction and the extraction of mineral salts such as KCl and borates.

The main environmental problems of the two towns are that the drinking water must be highly chlorinated because of its source and the absence of a sewerage system. In addition, San Pedro de Atacama lacks a garbage treatment facility.

Demographics. The population of the two towns are mostly of Aymara and Quechua ethnic native Chilean heritage, whose ancestors have lived in the area for 11,000 years (Nuñez-Atencio *et al.*, 1991). Volunteers were chosen from San Pedro de Atacama from those who were drinking at least some water from the water source having a high level of arsenic. These subjects were known from a previous study to have high arsenic levels in their urine (Hopenhayn-Rich *et al.*, 1996). Volunteers were also selected from Toconao, the control town. Sex and age matching was attempted (table 2). Six men were included from each town, but an equal number
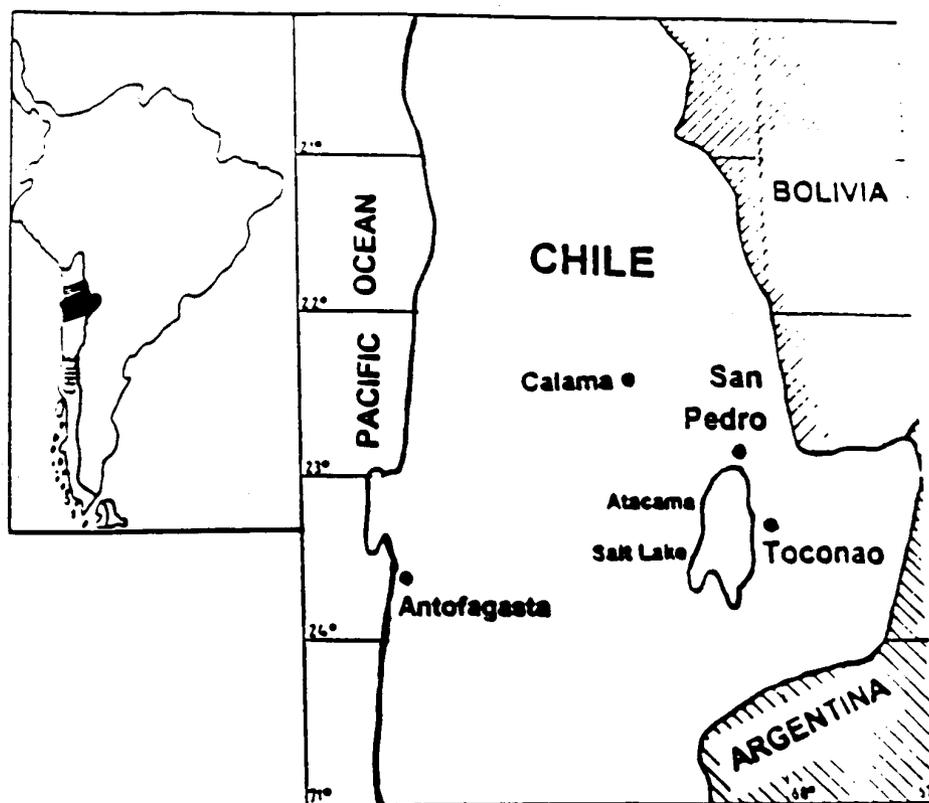
Fig. 1. Map showing locations of San Pedro de Atacama and Toconao in the Antofagasta Province of Chile (Hopenhayn-Rich *et al.*, 1996).

TABLE 2

Demographic factors associated with subjects

	San Pedro de Atacama	Toconao
Population	963	550
Subjects		
Admitted originally	15[a]	13[b]
Disqualified	2	2
Final number	13	11
Women	7	5
Men	6	6
Mean age, yr	42.5	44.5
Range	22–61	24–58
As, μg/l, in drinking water	593[c]	21[d]
Range, As, μg/l	500–720	10–40
As, μg/l in drinking water	528[e]	19.1[e]
Homes in town with triatomides[f]	3 %	2 %

[a] Two women elected not to continue during the overnight fast before DMPS administration.

[b] One man was disqualified because of a history of cardiovascular problems. One woman was disqualified because of vomiting during the overnight fast. Both were disqualified before DMPS administration.

[c] Average of 12 monthly collections and determinations during a 12-month period by the Health Services Laboratory of the Antofagasta District by use of atomic absorption spectrophotometry.

[d] Average of analyses for four different months by the Health Services Laboratory as above.

[e] Collected from tap at the time of this study and analyzed when the urine samples were analyzed.

[f] Vector for Chagas' Disease.

of women from each town was not obtained because there was an insufficient number of women volunteers from the control town. Other demographics are summarized in table 2.

Arsenic concentration of drinking water. Samples of San Pedro de Atacama and Toconao tap water, collected and analyzed for TotAs at the same time as the urine samples, contained 528 and 19.1 μg As/l, respectively (table 2). Recent reports by the Second District Government state that the drinking water of San Pedro de Atacama and Toconao contain 593 and 21 μg/l TotAs, respectively. The concentration of water removed from the San Pedro River water at the time of the study was determined to be 124 μg/l.

Concentration of arsenic species in the urine samples. The concentrations of TotAs (μg/g creatinine) excreted in the urine before and after the DMPS challenge were much greater in the subjects from San Pedro de Atacama than in those from the control town of Toconao at all time intervals (fig. 2). For both groups, the concentrations of each As species in the urine samples were the greatest during the 0- to 2-hr period (fig. 3), and did not return to the concentrations of the species found before the DMPS challenge.

Before DMPS administration, the urinary mean inorgAs concentration was approximately six times greater for the San Pedro de Atacama group than for the Toconao group. It was 7, 5, 7 and 9 times greater at 0 to 2, 2 to 4, 4 to 6 and 6 to 24 hr, respectively. The concentration values are included because toxicology is usually concerned with concentrations.

When the inorgAs concentrations before and 0 to 2 hr after the DMPS challenge were compared, DMPS increased the concentration 4.4- and 5.3-fold for the Toconao and San Pedro de Atacama subjects, respectively. For MMA, the concentration increased 11.9- and 10.2-fold, respectively, and for DMA, only 1.7- and 1.9-fold, respectively. There was good agreement between the summation of inorgAs + MMA + DMA with TotAs (table 3).

Amount of arsenic species in the urine samples. Of even greater interest than the concentrations were the *amounts* of
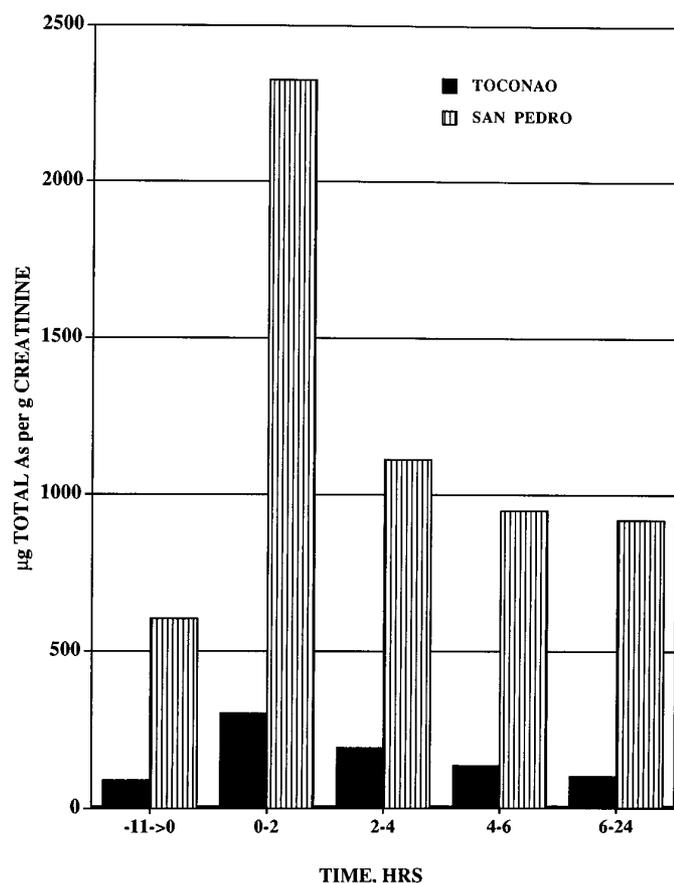
Fig. 2. Total arsenic concentration in urine before and after administration of 300 mg DMPS. DMPS was given p.o. at time 0. Error bars in this and other figures represent ± S.E.

Fig. 3. Concentration of arsenic species in the urine, μg/g creatinine, before and after 300 mg DMPS administration p.o. (A) San Pedro de Atacama subjects; (B) Toconao subjects.

the various arsenic species in the urine after the DMPS challenge because they are a better indicator of the body burden of arsenic. The mean TotAs in the urine of the San Pedro de Atacama subjects increased approximately 4-fold during the 2-hr period after DMPS administration, as compared with the preceding 2-hr period (fig. 4). When the San Pedro de Atacama and Toconao subjects were compared, there was a striking difference noted between the mean amount of TotAs excreted in the urine during all time periods (fig. 4).

In addition, the administration of DMPS resulted in a marked increase in the amount of urinary MMA of both the San Pedro and Toconao groups (fig. 5). There was approximately a 9-fold increase in urinary MMA during the 2-hr period after DMPS administration as compared with the previous 2 hr. For the same time periods, urinary inorgAs was increased about 5-fold and DMA less than 2-fold. From 2 to 6 hr after DMPS, the amount of inorgAs, MMA and DMA did not change to any great extent.

For the Toconao subjects, although the absolute amounts of arsenic species per 2-hr period were much less, the fold increases relative to the preceding time period were similar.

Percent of arsenic species in the urine. The percent inorgAs in the urine increased after DMPS administration (fig. 6). For MMA, it increased from 15% (San Pedro de Atacama) and 12% (Toconao) before DMPS administration to 42% for each group during the 0- to 2-hr period after DMPS. By the end of 6 hr, it had decreased to 28 and 24% for San Pedro de Atacama and Toconao groups, respectively. For
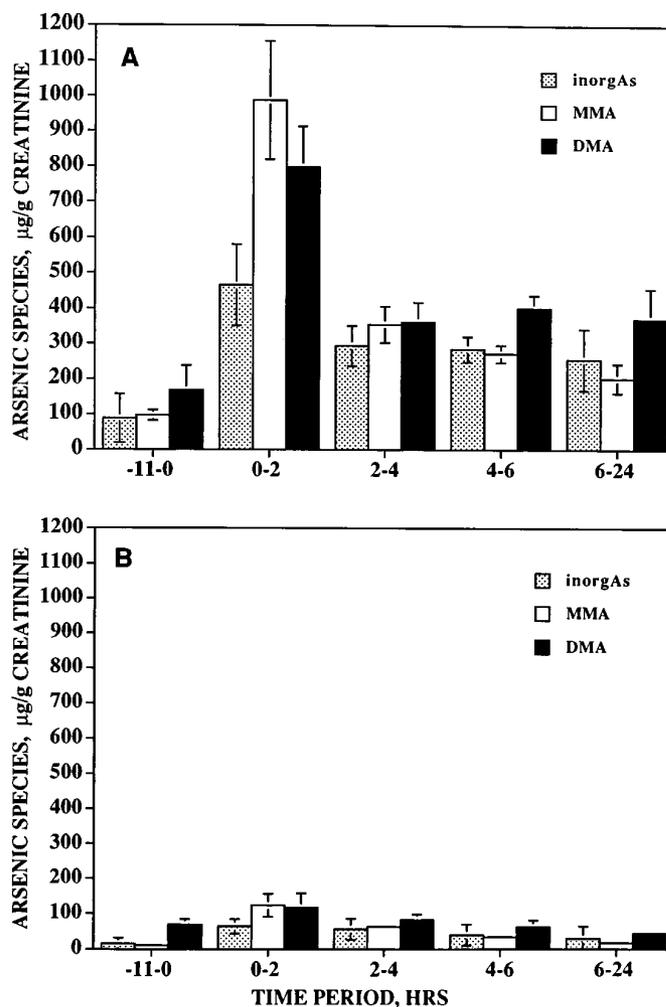
DMA, however, the percentage decreased after DMPS. Although DMPS administration resulted in significant changes in the percent of these various arsenic species excreted in the urine of both San Pedro de Atacama and Toconao subjects, the magnitude of the changes in relative percent were essentially the same for both groups (fig. 6).

The lowest MMA percentage for an individual of the Toconao group was 3.9% before DMPS, which became 39.4% for the 0 to 2 hr after DMPS administration. For the San Pedro de Atacama group, the lowest MMA percentage for an individual was 8.4%, which became 53.9% for the 0 to 2 hr after DMPS administration. Vahter *et al.* (1995a) reported that the mean percentage of MMA excretion in the urine of an unusual group of native Andean women, some drinking water high and others low in arsenic content, was 2.2 and 3.5%, respectively, with the range of 0.6 to 8.3 and 0.0 to 11.

The lowest DMA percentage of an individual of the Toconao group was 35.2% before DMPS, which became 26.2% 0 to 2 hr after DMPS administration *vs.* 42.6% and 12.1% for the individual with lowest percentage of the San Pedro de Atacama group.

Genotoxicity study. The prevalence of micronucleated cells among the San Pedro de Atacama subjects ($n = 9$) was 5-fold greater than that observed among the Toconao sub-

TABLE 3
Urinary concentrations of TotAs and sum of arsenic species for San Pedro de Atacama (SP) and Toconao (TOC) subjects

		Total As	InorgAs + MMA + DMA
		μg/g creatinine	
−11–0 hr			
SP	Mean	605	602
	SE	81	83
Toc	Mean	91	93
	SE	17	19
0–2 hr			
SP	Mean	2325	2222
	SE	258	294
Toc	Mean	305	304
	SE	79	83
2–4 hr			
SP	Mean	1111	1009
	SE	129	117
Toc	Mean	195	202
	SE	38	35
4–6 hr			
SP	Mean	950	956
	SE	57	66
Toc	Mean	138	136
	SE	32	30
6–24 hr			
SP	Mean	921	826
	SE	228	125
Toc	Mean	101	90
	SE	15	12

DMPS (300 mg) was given p.o. at time 0.

jects ($n = 8$) (table 4). By use of 2×2 contingency tables for χ^2 test, the occurrence of micronucleated cells (P < .01) and the frequency of positive subjects (P < .001) were found to be significantly associated with exposure. Smoking was not significantly associated with the frequency of micronuclei.

Other observations. There were no signs of arsenic toxicity as far as skin keratosis and ulcerations. Only one subject of the San Pedro de Atacama group had skin hypopigmentation, and she had only one such hypopigmented spot on her back.

Discussion

Several significant conclusions have resulted from this study. First, DMPS will mobilize arsenic in humans exposed to inorganic arsenic in their drinking water (figs. 2–6). There was a linear correlation (r = 0.89) between total urinary As excretion from 0 to 2 hr and 0 to 24 hr. This suggests that in the future a 0- to 2-hr urine sample after the DMPS challenge instead of a 0- to 24-hr sample might be representative and be collected. This, however, requires more experiments for confirmation.

Second, arsenic is stored in the human body. The increased urinary excretion of arsenic, especially in the San Pedro de Atacama subjects, after DMPS administration confirmed body retention of this toxic metalloid. One goal of this study was to determine whether there was storage of arsenic species in the human. The literature contains many statements that arsenic is not stored in the body. Farmer and Johnson (1990) indicated, however, that 40 to 60% of the arsenic is retained in the body.

Third, the use of DMPS gave a more accurate indication of the body burden of arsenic. The DMPS challenge test demonstrated that substantially more arsenic was excreted in the urine after DMPS administration and that the body burden of arsenic was greater than that indicated by the urinary

arsenic level found without the DMPS challenge. The urinary arsenic after a DMPS challenge, therefore, should have greater value for calculations involved in risk assessment of arsenic exposure.

The fourth significant point is that the San Pedro de Atacama subjects had a greater body burden of arsenic than those in the control town, as shown by a greater urinary excretion of arsenic species before and after DMPS administration, than did the Toconao subjects. This is not surprising because the water they generally drink has a much higher level of arsenic (593 *vs.* 21 μg As/l). The amount of various arsenic species in the urine may not have decreased to that of the pre-DMPS level because the subjects were not followed for a long enough period of time.

An important result of this study was that DMPS administration resulted in a marked change in the amounts of arsenic species being excreted in the urine (figs. 5 and 6). The most striking change was for MMA. The %MMA increased (fig. 6) to an extent never seen in any previously reported study in humans. Previous reports (Offergelt *et al.*, 1992; Yamauchi *et al.*, 1992; Hopenhayn-Rich *et al.*, 1993) indicated that the usual percentage of MMA in human urine has a range of about 10 to 20%. In the San Pedro de Atacama group, before DMPS administration, the MMA was 15% of the urinary arsenic species. During the 2-hr period after DMPS administration, it became 42%. The increase in MMA% appeared to be accompanied by a substantial decrease in DMA%. Between 2 and 4 hr after DMPS administration, the MMA% in the urine began to decrease and the inorgAs began to increase. The percent of a given arsenic species in the urine did not return to the pre-DMPS levels during the collection periods studied. The ratios of the arsenic species found in the urine after DMPS administration were independent of the arsenic body burden (fig. 6). The changes in the percent of urinary arsenic species for the Toconao group were very similar to those found for the San Pedro de Atacama group (fig. 6).

There are several possible explanations for this increase in MMA percentage. Some involve the methyltransferases that are involved in the metabolism of inorganic arsenic (for reviews, see Aposhian, in press; Aposhian *et al.*, in press; Styblo *et al.*, 1995a). These enzymes have received increasing attention recently because different mammals show striking species variation and polymorphism (Zakharyan *et al.,* 1995, 1996; Vahter *et al.*, 1995a, b; Healy *et al.*, 1997). 1) DMPS may inhibit the methyltransferase activity that methylates MMA to produce DMA, which results in the accumulation of MMA in the body and urine with less DMA being available for urinary excretion. But DMPS at concentrations of 1 to 10 μM did not inhibit a 2800-fold purified rabbit liver arsenite or MMA methyltransferase *in vitro* (Zakharyan, personal communication) assayed in the presence of 3.3 mM GSH under the conditions described by Zakharyan *et al.* (1995). Neither could 10 to 40 μM DMPS replace GSH as a required thiol in these assays. These DMPS concentrations are in the range of those found in human blood after a DMPS challenge test (Maiorino *et al.*, 1991). Buchet and Lauwerys (1988), however, reported that 0.05 to 0.5 mM DMPS almost completely abolished the methylation reaction by a rat liver cytosol preparation, but it was not clear whether the assay was performed with or without GSH. 2) DMPS with its two thiol groups may have chemically reduced arsenate to arsen-
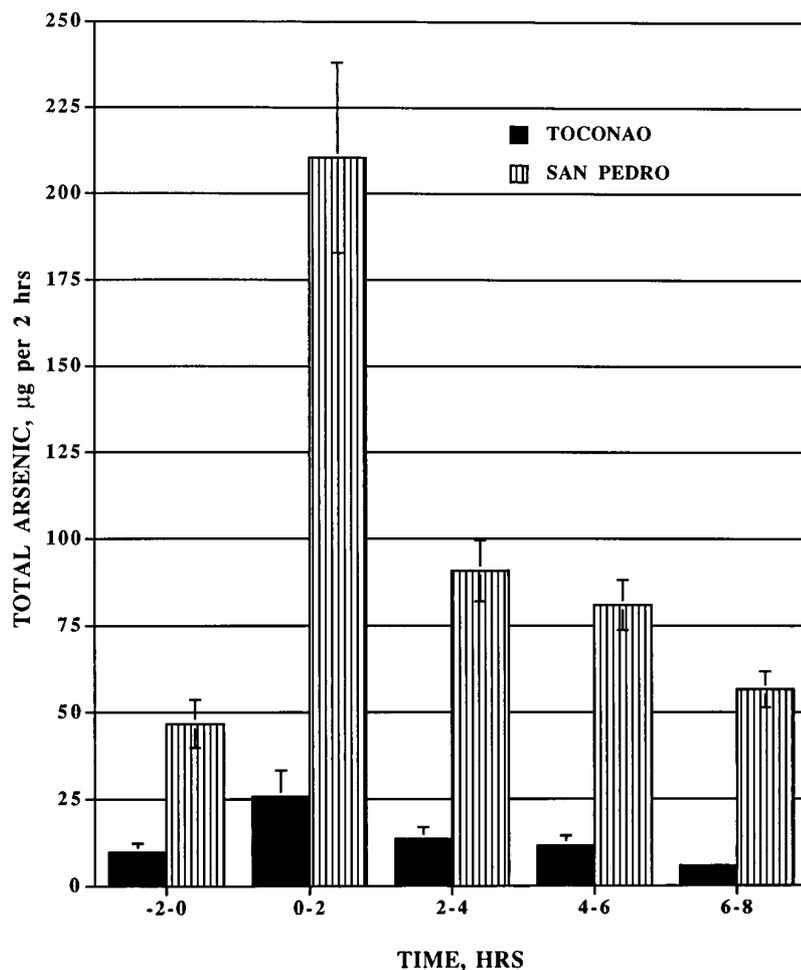
Fig. 4. Total arsenic excreted in the urine before and after administration of 300 mg DMPS p.o. DMPS was given at time 0. Although urine was collected overnight (−11 to 0 hr) and for 6 to 24 hr, the total amount of arsenic excreted for these times was divided by 5.5 and 9 to make the data comparable with the 2-hr urine collection periods of 0 to 2, 2 to 4 and 4 to 6 hr.

ite *in vivo* and thus more MMA was synthesized and made available for excretion. 3) The reducing environment produced by DMPS may have stimulated in some manner the mechanisms involved in the urinary excretion of MMA but not DMA. 4) MMA containing As^{+++++} may be stored in the human body before excretion. If so, DMPS may have reduced it to MMA containing As^{+++}, which appears to be more chelatable by DMPS, or the DMPS may have chelated this elusive species of MMA containing As^{+++} and thus increased its excretion. Such a trivalent form has been postulated but never isolated from *in vivo* or *in vitro* studies. In support of this, to some extent, is that when the urine samples were not first digested with 2 M HCl, the summation of the separately determined inorgAs + MMA + DMA by the method of Crecelius (1986) was about 60% lower than the experimentally determined total arsenic. It is for this reason that the arsenic species analyses of this paper were performed after digestion with 2 M HCl. With this relatively gentle digestion procedure, there was good agreement between the summation of inorgAs + MMA + DMA with TotAs. These irregularities did not occur when −11 to 0 hr urine samples were analyzed, because they were collected before DMPS had been administered. In addition, when four of the urine samples of this study were analyzed by high-performance liquid chromatography-inductively coupled plasma-mass spectrometry, a broad new unidentified peak overlapping the MMA peak was found in urine samples collected after DMPS administration

(K. Irgolic and W. Goessler, personal communication). This peak disappeared on acid treatment of the urine. Whether this new peak is or is not the DMPS-As chelate has not been established. No other information about the structure of this unidentified peak is currently available.

It should be noted that the percentage of inorgAs also increased after DMPS administration, but the rate of increase and the magnitude of the increase were not as great as that for MMA. An inorgAs change of this magnitude is unusual in humans but has been reported also by Del Razo *et al.* (1997).

The subjects for the present study were part of a larger group of 122 people from San Pedro de Atacama and 98 from Toconao who had been studied previously by Hopenhayn-Rich *et al.* (1996). In the previous study, the MMA/DMA ratio was 1.5 times greater in the group drinking water with a high arsenic content. This was true also in the present study for the −11- to 0-hr urine, that is, before DMPS administration.

When DMPS was given intramuscularly to rabbits after a subcutaneous injection of arsenite, an increased MMA but not DMA excretion was also noted (Maiorino and Aposhian, 1985), which again demonstrates the value of the rabbit as a model for the human's metabolic processing of inorganic arsenic as has been suggested previously (Maiorino and Aposhian, 1985; Vahter, 1983).

An additional result of this study has genotoxic importance. This South American group had a similar genotoxic response in oral epithelial cells (table 4) to that found in
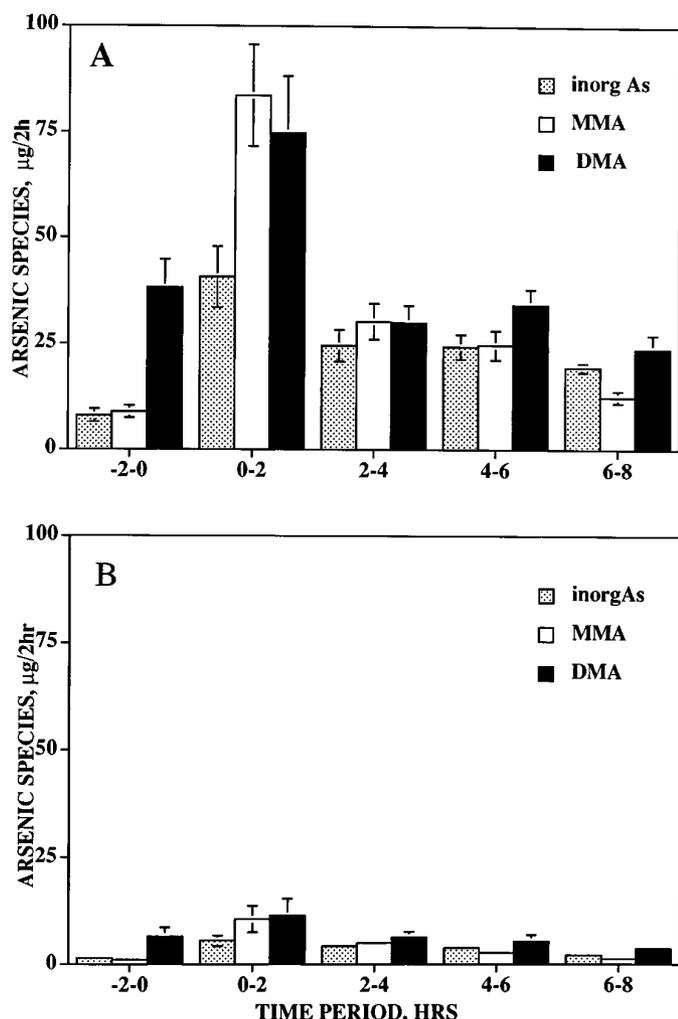
Fig. 5. Urinary excretion of inorganic arsenic, MMA and DMA during 2-hr time periods before and after administration of 300 mg DMPS p.o. (A) San Pedro de Atacama subjects; (B) Toconao (control) subjects. DMPS was given at time 0.

Fig. 6. Arsenic species expressed as a percentage of urinary TotAs at time periods before and after DMPS administration. DMPS was given p.o. at time 0. Percentages for the Toconao group are in parentheses.

TABLE 4

Frequency of micronuclei per 1000 buccal cells

Subject	From Toconao	From San Pedro de Atacama
1	0	0.7
2	0	1
3	0	2.6
4	2.5	0
5	0.9	4.8
6	0.6	0
7	0	1.9
8	0	2
9	–	2.5
10	–	1.7
Mean ± S.E.	0.30 ± 0.30	1.72 ± 0.45*

* $P < .05$.

buccal smears from 33 individuals in northern Mexico who had been chronically exposed to 396 to 435 μg As/l in their drinking water (Gonsebatt *et al.*, in press). These results, and the fact that micronuclei frequencies in exfoliated cells have been validated as tissue-specific dosimeters of carcinogen exposure in humans (Rosin, 1992), strengthens the value of this assay for the demonstration of early biological effects of arsenic exposure and could be an aid in risk assessment of known or suspected arsenic exposure.

DMPS is not a new drug, even though it is still an investigational drug in the United States. It was developed in the 1950s in the former Soviet Union and became an official drug in the Soviet physician's armamentarium in 1958 (Aposhian, 1983). It was introduced into the Western world in 1978. Since then, it has had wide use, especially in Germany, as a chelating agent for both the diagnosis and mobilization of inorganic mercury in the body (for reviews, see Aposhian, 1983; Aposhian *et al.*, 1995; Kemper *et al.*, 1990; Aaseth *et al.*, 1995). This use has been extensive because of the concerns about humans exposed to elemental mercury emitted from dental amalgams *in vivo* (Lorscheider *et al.*, 1995; Aposhian *et al.*, 1992) and dental personnel exposed occupationally to mercury (Gonzalez-Ramirez *et al.*, 1995).
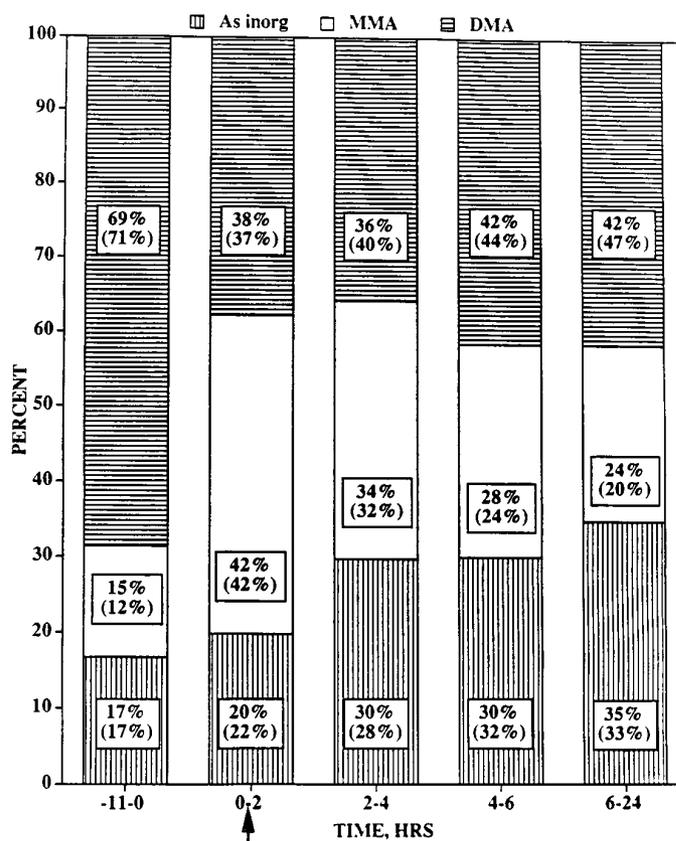
Although the present study has yielded several new and important contributions to our knowledge about arsenic exposure of humans *via* drinking water, and the usefulness of DMPS as a mobilizing agent for arsenic, especially for MMA, the early use of DMPS to decrease the As body burden of such subjects would appear warranted, to decrease possible cancer and genotoxicity risks.

References

AASETH, J., JACOBSEN, D., ANDERSEN, O. AND WICKSTROM, E.: Treatment of mercury and lead poisonings with dimercapto succinic acid and sodium dimercaptopropanesulfonate: A review. Analyst **120:** 853–854, 1995.

APOSHIAN, H. V.: Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu. Rev. Pharmacol. Toxicol., in press, 1997.

APOSHIAN, H. V.: DMSA and DMPS-water soluble antidotes for heavy metal poisoning. Annu. Rev. Pharmacol. Toxicol. **23:** 193–215, 1983.

APOSHIAN, H. V. AND APOSHIAN, M. M.: Meso-2,3-dimercaptosuccinic acid: Chemical, pharmacological and toxicological properties of an orally effective chelating agent. Annu. Rev. Pharmacol. Toxicol. **30:** 279–306, 1990.

APOSHIAN, H. V., BRUCE, D. C., ALTER, W., DART, R. C., HURLBUT, K. M. AND APOSHIAN, M. M.: Urinary mercury after administration of 2,3-dimercaptopropane-1-sulfonic acid: correlation with dental amalgam score. FASEB J. **6:** 2472–2476, 1992.

APOSHIAN, H. V., MAIORINO, R. M., GONZALEZ-RAMIREZ, D., ZUNIGA-CHARLES, M., XU, Z., HURLBUT, K. M., JUNCO-MUNOZ, P., DART, R. C. AND APOSHIAN, M. M.: Mobilization of heavy metals by newer, therapeutically useful chelating agents. Toxicology **97:** 23–38, 1995.

APOSHIAN, H. V., ZAKHARYAN, R. A., WU, Y., HEALY, S. AND APOSHIAN, M. M.: Enzymatic methylation of arsenic compounds: II. An overview. *In* Arsenic Exposure and Human Health II, ed. by C. Abernethy, R. Calderon, W. Chappell, Chapman & Hall, London, in press, 1997.

BUCHET, J. P. AND LAUWERYS, R.: Role of thiols in the in-vitro methylation of inorganic arsenic by rat liver cytosol. Biochem. Pharmacol. **37:** 3149–3153, 1988.

CEBRIAN, M. E., ALBORES, A., AGUILAR, M. AND BLAKELY, E.: Chronic arsenic poisoning in the north of Mexico. Hum. Toxicol. **2:** 121–133, 1983.

CHATTERJEE, A., DAS, D., MANDAL, B. K., CHOWDHURY, T. R., SAMANTA, G. AND CHAKRABORTY, D.: Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. Part I. Arsenic species in drinking water and urine of the affected people. Analyst **120:** 643–650, 1995.

CHEN, C. J., CHUANG, Y. C., LIN, T. M. AND WU, H. Y.: Malignant neoplasms among residents of a Blackfoot disease-endemic area in Taiwan: high-arsenic artesian well water and cancers. Cancer Res. **45:** 5895–5899, 1985.

CHEN, S. L., YEH, S. J., YANG, M. H. AND LIN, T. H.: Trace element concentration and arsenic speciation in the well water of a Taiwan area with endemic Blackfoot Disease. Biol. Trace Elem. Res. **48:** 263–274, 1995.

COX, D. H.: Arsine evolution-electrothermal atomic absorption method for the determination of nanogram levels of total arsenic in urine and water. J. Anal. Toxicol. **4:** 207–211, 1980.

CRECELIUS, E.: Speciation of Selenium and Arsenic in Natural Waters and Sediments, Vol. 2: Arsenic Speciation. EPRI EA-4641s, 1986.

DEL RAZO, L. M., ARELLANO, M. A. AND CEBRIAN, M. E.: The oxidation states of arsenic in well water from a chronic arsenicism area of northern Mexico. Environ. Poll. **64:** 143–153,1990.

DEL RAZO, L. M., GARCIA-VARGAS, G. G, VARGAS, H., ALBORES, A., GONSEBATT, M. E., MONTERO, R., OSTROSKY-WEGMAN, P., KELSH, M. Y. AND CEBRIAN, M. E.: Altered profile of urinary arsenic metabolites in adults with chronic arsenism. A pilot study. Arch. Toxicol., in press, 1997.

FARMER, J. G. AND JOHNSON, L. R.: Assessment of occupational exposure to inorganic arsenic based on urinary concentrations and speciation of arsenic. Br. J. Ind. Med. **47:** 342–348, 1990.

GONSEBATT, M. E., VEGA, L., SALAZAR, A. M., MONTERO, R., GUZMAN, P., BLAS, J., DEL RAZO, L. M., GARCIA-VARGAS, G., ALBORES, A., CEBRIAN, M. E., KELCH, M. AND OSTROSKY-WEGMAN, P.: Clastogenicity of arsenic exposure. Mutat. Res., in press, 1997.

GONZALEZ-RAMIREZ, D., MAIORINO, R. M., ZUNIGA-CHARLES, M., XU, Z., HURLBUT, K. M., JUNCO-MUNOZ, P., APOSHIAN, M. M., DART, R. C., DIAZ GAMA, J. H., ECHEVERRIA, D., WOODS, J. S. AND APOSHIAN, H. V.: Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans: II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey, Mexico. J. Pharmacol. Exp. Ther. **272:** 264–274, 1995.

GUHA MAZUMDER, D. N., CHAKRABORTY, A. K., GHOSE, A., GUPTA, J. D., CHAKRABORTY, D. P. AND DEY, S. B.: Chronic arsenic toxicity from drinking water in rural West Bengal. Bull. WHO **66:** 499–506, 1988.

HAGMAR, L., BROGGER, A., HANSTEEN, I-L., HEIM, S., HOGSTED, B., KNUDSEN, L., LAMBERT, B., LINNAINMAA, K., MITELMAN, F., NORDENSON, I., REUTERWALL, C., SALORNAA, S., SKERFVING, S. AND SORSA, M.: Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Res. **54:** 2919–2922, 1994.

HEALY, S. M., ZAKHARYAN, R. A. AND APOSHIAN, H. V.: Enzymatic methylation of arsenic compounds: IV. In vitro and in vivo deficiency of the methylation of arsenite and monomethylarsonic acid in the guinea pig. Mutat. Res., in press, 1997.

HOPENHAYN-RICH, C., BIGGS, M. L., KALMAN, D. A., MOORE, L. E. AND SMITH, A. H.: Arsenic methylation patterns before and after changing from high to lower concentrations of arsenic in drinking water. Environ. Health Perspect. **104:** 1200–1207, 1996.

HOPENHAYN-RICH, C., SMITH, A. H. AND GOEDEN, H. M.: Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. Environ. Res. **60:** 161–177, 1993.

IARC: Arsenic and arsenic compounds. *In* IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Metals and Metallic Compounds. World Health Organization, Lyon, France **23:** 39–141, 1980.

KEMPER, F. H., JEKAT, F. W., BERTRAM, H. P. AND ECKARD, R.: New chelating agents. *In* Basic Science in Toxicology, Proceedings of the 5th International Congress of Toxicology, England 1989, ed. by G. N. Volans, J. Sims, F. M. Sullivan and P. Turner, pp. 523–546, Taylor & Francis Ltd, London, 1990.

LORSCHEIDER, F. L., VIMY, M. J. AND SUMMERS, A. O.: Mercury exposure from "silver" tooth fillings: emerging evidence questions a traditional dental paradigm. FASEB J. **9:** 504–08, 1995.

MAIORINO, R. M. AND APOSHIAN, H. V.: Dimercapto metal-binding agents influence the biotransformation of arsenite in the rabbit. Toxicol. Appl. Pharmacol. **77:** 240–250, 1985.

MAIORINO, R. M., CARTER, D. E. AND APOSHIAN, H. V.: Determination and metabolism of dithiol chelating agents. XII. Metabolism and pharmacokinetics of sodium 2,3-dimercaptopropane-1-sulfonate in humans. J. Pharmacol. Exp. Ther. **259:** 808–814, 1991.

MAIORINO, R. M., GONZALEZ-CHARLES, M., XU, Z., HURLBUT, K. M., APOSHIAN, M. M., DART, R. C., WOODS, J. S., OSTROSKY-WEGMAN, P., GONSEBATT, M. E. AND APOSHIAN, H. V.: Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans. III. Urinary mercury after exposure to mercurous chloride. J. Pharmacol. Exp. Ther. **277:** 938–944, 1996.

NATIONAL RESEARCH COUNCIL REPORT: Medical and Biologic Effects of Environmental Pollutants, Arsenic National Academy of Sciences, Washington, DC, 1977.

NUÑEZ-ATENCIO, L. N., HILL, H. G. AND MARTÍNEZ, A. L.: Guía Museo Arqueológico, Universidad Católica del Norte Chile. San Pedro de Atacama (II Región): Instituto de Invetigaciones Arqueológicas y Museo R. P. Gustavo Le Paige S. J., 1991.

OFFERGELT, J. A., ROELS, H., BUCHET, J. P., BOECKX, M. AND LAUWERYS, R.: Relation between airborne arsenic trioxide and urinary excretion of inorganic arsenic and its methylated metabolites. Br. J. Ind. Med. **49:** 387–393, 1992.

ROSIN, M. P.: Micronuclei as intermediate end points in intervention. *In* The Biology and Prevention of Aerodigestive Tract Cancers, ed. by G. R. Newell and W. K. Wong, pp. 95–105, Plenum Press, New York, 1992.

SANCHA, A. M., VEGA, F., VENTURINO, H., FUENTES, S., SALAZAR, A. M., MORENO, V., BARON, A. M. AND RODRIGUEZ, D.: The arsenic health problem in northern Chile evaluation and Control. A case study preliminary report. *In* Procceedings of the International Seminar. Arsenic in the Environment and Its Incidence on Health, pp. 187—202, Universidad de Chile, Santiago, Chile, 1992.

STYBLO, M., DELNOMDEDIEU, M. AND THOMAS, D.: Biological mechanisms and toxicological consequences of the methylation of arsenic. *In* Toxicology of Metals-Biochemical Aspects, Handbook of Experimental Pharmacology, v. 115, ed. by M. G. Cherian and R. A. Goyer, pp. 407–433, Springer Verlag, Berlin, 1995a.

STYBLO, M., YAMAUCHI, H. AND THOMAS, D.: Comparative in vitro methylation of trivalent and pentavalent arsenicals. Toxicol. Appl. Pharmacol. **135:** 172–178, 1995b.

TOLBERT, P. E., SHY, C. M. AND ALLEN, J. W.: Micronuclei and other anomalies in buccal smears: methods development. Mutat. Res. **271:** 69–77, 1992.

TSENG, C. H., CHONG, C. K., CHEN, C. J. AND TAI, T. Y.: Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in Blackfoot Disease endemic villages in Taiwan. Atherosclerosis **120:** 125–133, 1996.

TSENG, W. P.: Effects and dose-response relationships of skin cancer and Blackfoot Disease with arsenic. Environ. Health Perspect. **19:** 109–119, 1977.

VAHTER, M.: Metabolism of inorganic arsenic in relation to chemical form and animal species, Ph.D. Thesis, Karolinska Institute and National Institute of Environmental Medicine, Stockholm, Sweden, 1983.

VAHTER, M., CONCHA, G., NERMELL, B., NILSSON, R., DULUOT, F. AND NATARAJAN, A.: A unique metabolism of inorganic arsenic in native Andean women. Eur. J. Pharmacol. **293:** 455–462, 1995a.

VAHTER, M., COUCH, R., NERMELL, B. AND NILSSON, R.: Lack of methylation of inorganic arsenic in the chimpanzee. Toxicol. Appl. Pharmacol. **133:** 262–268, 1995b.

VAHTER, M. AND LIND B.: Concentrations of arsenic in urine of the general population in Sweden. Sci. Total Environ. **54:** 1–12, 1986.

WORLD HEALTH ORGANIZATION: Guidelines for drinking-water quality. Vol. 1. Recommendations. World Health Organization, Geneva, 1996.

YAMAUCHI, H., TAKAHASHI, K., MASHIKO, M., SAITOH, J. AND YAMAMURA, Y.: Intake of different chemical species of dietary arsenic by Japanese, and their blood and urinary arsenic levels. Appl. Organomet. Chem. **6:** 383–388, 1992.

ZAKHARYAN, R. A., WILDFANG, E. AND APOSHIAN, H. V.: Enzymatic methylation of arsenic compounds: III. The marmoset and tamarin, but not the rhesus, monkey are deficient in methyltransferases that methylate inorganic arsenic. Toxicol. Appl. Pharmacol. **140:** 77–84, 1996.

ZAKHARYAN, R. A., WU, Y., BOGDAN, G. M. AND APOSHIAN, H. V.: Enzymatic methylation of arsenic compounds. I: Assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem. Res. Toxicol. **8:** 1029–1038, 1995.

Send reprint requests to: Dr. H. Vasken Aposhian, Department of Molecular and Cellular Biology, Life Sciences South Bldg, Rm. 444, P.O. Box 210106, University of Arizona, Tucson, AZ 85721-0106.