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Metabolism of Arsenic in Primary Cultures of Human and Rat Hepatocytes

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The liver is considered a major site for methylation of inorganic arsenic (iAs). However, there is little data on the capacity of human liver to methylate iAs. This work examined the metabolism of arsenite (iAs^{III}), arsenate (iAs^{V}), methylarsine oxide ($MAs^{III}O$), methylarsonic acid (MAs^{V}), dimethylarsinous acid ($DMAs^{III}$), and dimethylarsinic acid ($DMAs^{V}$) in primary cultures of normal human hepatocytes. Primary rat hepatocytes were used as methylating controls. iAs^{III} and $MAs^{III}O$ were metabolized more extensively than iAs^{V} and MAs^{V} by either cell type. Neither human nor rat hepatocytes metabolized $DMAs^{III}$ or $DMAs^{V}$. Methylation of iAs^{III} by human hepatocytes yielded methylarsenic (MAs) and dimethylarsenic (DMAs) species; $MAs^{III}O$ was converted to DMAs. The total methylation yield (MAs and DMAs) increased over the range of 0.1 to 4 μM iAs^{III}. However, DMAs production was inhibited by iAs^{III} in a concentration-dependent manner, and the DMAs/MAs ratio decreased. iAs^{III} (10 and 20 μM) inhibited both methylation reactions. Inhibition of DMAs synthesis resulted in accumulation of iAs and MAs in human hepatocytes, suggesting that dimethylation is required for iAs clearance from cells. Methylation capacities of human hepatocytes obtained from four donors ranged from 3.1 to 35.7 pmol of iAs^{III} per 10^6 cells per hour and were substantially lower than the methylation capacity of rat hepatocytes (387 pmol of iAs^{III} per 10^6 cells per hour). The maximal methylation rates for either rat or human hepatocytes were attained between 0.4 and 4 μM iAs^{III}. In summary, (i) human hepatocytes methylate iAs, (ii) the capacities for iAs methylation vary among individuals and are saturable, and (iii) moderate concentrations of iAs inhibit DMAs synthesis, resulting in an accumulation of iAs and MAs in cells.

Introduction

Biomethylation is a major metabolic pathway for inorganic arsenicals (iAs)[1] in humans and in most animal species (*1*). Both pentavalent iAs, arsenate (iAs^{V}), and trivalent iAs, arsenite (iAs^{III}), are metabolized to yield mono-, di-, and possibly trimethylated metabolites. This metabolic pathway involves two types of reactions: (i) the reduction of pentavalent arsenicals to trivalent species and (ii) the oxidative methylation of trivalent arsenicals to pentavalent methylated species. Thus, pentavalent and trivalent methylated arsenicals, methylarsonic acid (MAs^{V}), methylarsonous acid (MAs^{III}), dimethylarsinic acid ($DMAs^{V}$), dimethylarsinous acid ($DMAs^{III}$), and trimethylarsinoxide ($TMAs^{V}O$), are intermediaries or final products in pathway I (*2*):

$$iAs^{V} \rightarrow iAs^{III} \rightarrow MAs^{V} \rightarrow MAs^{III} \rightarrow DMAs^{V} \rightarrow DMAs^{III} \rightarrow TMAs^{V}O \quad \text{(I)}$$

Because MAs^{V}, $DMAs^{V}$, and $TMAs^{V}O$ are less acutely toxic than iAs, methylation is commonly considered a mechanism of detoxification (*3*).

Most data on qualitative and quantitative aspects of iAs methylation have been obtained from in vitro studies using rat liver cytosol (*4*−*6*), primary rat hepatocytes (*7*), or a partially purified arsenic methyltransferase from rabbit liver (*8*). Arsenic methyltransferase activity was not detected in one sample of human liver (*9*). There are

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[1]Abbreviations: iAs, inorganic arsenic; iAs^{V}, arsenate; iAs^{III}, arsenite; MAs^{V}, methylarsonic acid; MAs^{III}, methylarsonous acid; $MAs^{III}O$, methylarsine oxide; $DMAs^{V}$, dimethylarsinic acid; $DMAs^{III}$, dimethylarsinous acid; $DMAs^{III}GS$, complex of $DMAs^{III}$ with GSH; MAs, methylarsenic species (MAs^{V} or MAs^{III}); DMAs, dimethylarsenic species ($DMAs^{V}$ or $DMAs^{III}$); $TMAs^{V}O$, trimethylarsine oxide; FBS, fetal bovine serum; HG−AAS, hydride generation−atomic absorption spectrometry; MTT, thiazolyl blue; AMR, apparent methylation rate.

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Table 1. Characteristics of Human Livers Used for Preparation of Hepatocytes

donor	sex	age (years)	race	origin/surgical procedure
1	male	28	white	transplant donor
2	female	66	white	resection[a]
3	male	66	white	resection[a]
4	male	67	white	resection[a]

[a] Normal liver tissue obtained from resections was used for perfusion.

no data that describe the extent or pattern for the methylation of arsenic in other human tissues. The work reported here examined the metabolism of iAs and MAs in primary cultures of normal human hepatocytes. Primary rat hepatocytes were used in parallel as methylating controls. Trivalent arsenicals but not pentavalent arsenicals were efficiently methylated by both human and rat hepatocytes. The methylation rate and DMAs/MAs ratio in human hepatocytes were 1−2 orders of magnitude lower than those observed in rat cells. In either cell line, the methylation reactions, particularly DMAs synthesis, were inhibited by high concentrations of iAs^{III}.

Experimental Procedures

Caution: *Inorganic arsenic is classified as a human carcinogen (10). The toxic and carcinogenic effects of trivalent methylated arsenicals have not been systematically examined.*

Arsenicals. iAs^{V} and iAs^{III} (sodium salts) were purchased from Sigma (St. Louis, MO). MAs^{V} (sodium salt) was obtained from Chem Service (West Chester, PA) and $DMAs^{V}$ from Strem (Newburyport, MA). Methylarsine oxide ($MAs^{III}O$) and a complex of $DMAs^{III}$ with GSH ($DMAs^{III}GS$) were synthesized as previously described (*2, 11*). The identity and purity of these compounds were confirmed by MS and ^1H NMR. Radiolabeled [^{73}As]iAs^{V} (13.3 Ci/mg of As) was purchased from Los Alamos Meson Production Facility (Los Alamos, NM). [^{73}As]iAs^{III} was prepared from [^{73}As]iAs^{V} as previously described (*6, 12*).

Isolation and Treatment of Human and Rat Hepatocytes. Human hepatocytes were isolated from normal liver samples (perfused with Belzer's solution and stored at 4 °C for up to 24 h) or from fresh normal biopsies obtained at Memorial Hospital, University of North Carolina at Chapel Hill. Characteristics of human donors are summarized in Table 1. Hepatocytes were isolated using a two-step collagenase perfusion procedure based on that of Strom and co-workers (*13*). Cell viability (determined by trypan blue exclusion) ranged from 80 to 90%. Hepatocytes were plated in 24-well plates coated with collagen-I (Becton Dickinson, Bedford, MA) in DMEM (Gibco BRL, Grand Island, NY) with 5% fetal bovine serum (FBS, Gibco BRL), 4 μg/mL insulin (Sigma), and 1 μM dexamethasone (Sigma). After attachment for 3−4 h, this medium was replaced with William's medium E supplemented with 2 mM glutamine (Sigma), 100 units/mL penicillin (Sigma), 100 μg/mL streptomycin (Sigma), 5 μg/mL bovine insulin, 5 μg/mL human transferrin (Sigma), 5 ng/mL sodium selenite (Sigma), and 0.5 μM dexamethasone. The integrity of human hepatocytes in cultures was assessed by morphological and functional criteria, including cellular GSH contents and basal and induced activities of P450 (CYP3A4) (see the Supporting Information).

Rat hepatocytes were prepared at the Advanced Cell Technologies Core, University of North Carolina at Chapel Hill, from adult male Fischer 344 rats using a two-step perfusion technique (*14*). Cell viability ranged from 90 to 97%. Hepatocytes were cultured in collagen-I-coated 24-well plates in William's medium E with the same additives used for human hepatocytes. All primary cultures were maintained at 37 °C in a humidified chamber in a 95% air/5% CO_2 atmosphere. Cells were cultured for 48 h before being used, and media were changed daily. Cells were incubated with [^{73}As]iAs^{III}, [^{73}As]iAs^{V} (0.1−20 μM), or stable MAs or DMAs species (1 μM) for up to 48 h without changing media. Sodium arsenite or sodium arsenate (Sigma) was added to radiolabeled compounds as required.

Analysis of Arsenic Metabolites. Radiolabeled metabolites were analyzed in trypsinized cells and in media using previously described techniques (*6, 15*). Metabolites in cells incubated with stable arsenicals were assessed by hydride generation−atomic absorption spectrophotometry (HG−AAS). Here, combined cells and medium were solubilized in 2 M HCl at 80 °C for 3 h. Arsenic metabolites were analyzed using a Perkin-Elmer 5100 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT) (*16*). The accuracy of analysis was assessed by the method of additions with known amounts of various arsenicals. Recoveries ranged from 92 to 107%; coefficients of variation ranged between 3 and 11%. Because the analytical methods used here cannot determine the original oxidation states of arsenic metabolites, the valency of metabolites in cells or media cannot be specified. Hence, arsenic metabolites are generically termed iAs, MAs, and DMAs.

Examination of the Cytotoxicity of iAs. Cell viability was determined using a thiazolyl blue (MTT) assay (*17*) following a 24 h exposure to arsenicals. Cells treated with Triton X-100 (Sigma) were used as positive controls.

Results

Metabolism of iAs in Human and Rat Hepatocytes. Figure 1 shows metabolic profiles in cells and medium for human (donor 2) and rat hepatocytes exposed to 0.1 μM iAs^{III} for up to 48 and 24 h, respectively. Cellular contents of iAs in human hepatocytes peaked after exposure for 1 h and declined steadily thereafter. In contrast, cellular contents of MAs and DMAs peaked after exposure for 9 and 24 h, respectively. The uptake and release of metabolites from cells were monitored by speciation of arsenic in the culture medium. The iAs content in medium declined steadily throughout the incubation. Both MAs and DMAs were undetectable in medium over the first 6 h of exposure. At later time points, MAs contents of the medium increased only slightly. DMAs contents of medium increased steadily throughout the 48 h exposure and ultimately accounted for 60% of the arsenic in the culture.

In rat hepatocytes, the cellular contents of iAs, MAs, and DMAs were highest after exposure for 2 h and then rapidly declined. DMAs was the major cellular metabolite over the first 12 h of exposure; MAs was always a minor cellular metabolite. The dynamic changes in cellular contents of arsenicals were reflected by the pattern of metabolites in the medium. The iAs contents of medium declined rapidly over the first 12 h of exposure. The decline of iAs in medium paralleled a rise in DMAs. Beyond 2 h of exposure, DMAs was the major metabolite in medium, eventually accounting for more than 90% of the arsenic in the culture. In contrast, in human and rat hepatocytes incubated with iAs^{V} (0.1 μM for up to 48 h), the total methylation yield (DMAs and MAs) did not exceed 3% (data not shown). In iAs^{V}-exposed cells, less than 6% of total arsenic was associated with cells as compared with up to 30% in cells exposed to iAs^{III}. Methylated metabolites were not found in medium incubated for up to 24 h with either iAs^{III} or iAs^{V} in the absence of cells.

Metabolism of MAs and DMAs in Human and Rat Hepatocytes. The capacity of human and rat hepatocytes to methylate or demethylate MAs and DMAs was tested by exposing cells to 1 μM $MAs^{III}O$, 1 μM MAs^{V}, 1

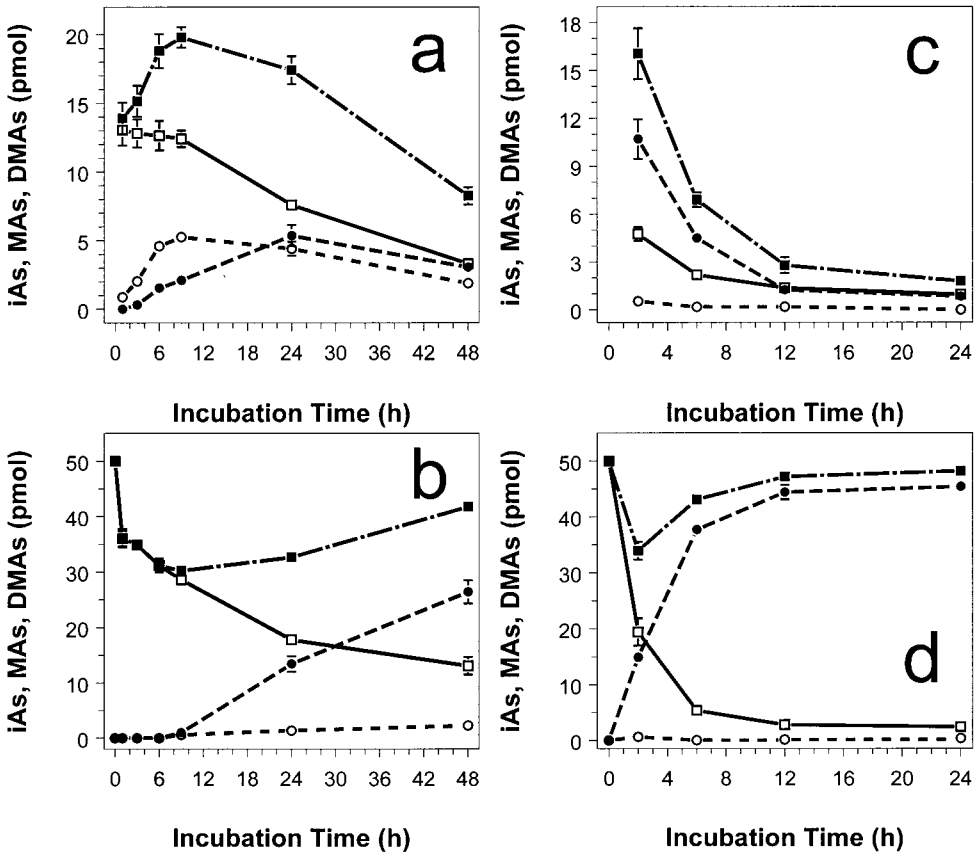

Figure 1. Metabolism of 0.1 μM iAs^{III} in primary cultures of human (a and b) and rat (c and d) hepatocytes, with (a and c) arsenic metabolites in cells and (b and d) arsenic metabolites in medium: iAs (□), MAs (○), DMAs (●), and total arsenic (■). Human cells (2×10^5) and rat cells (10^5) were plated per well in 0.5 mL of medium. Each point and error bar represents the mean ± SD for four wells.

Table 2. Metabolites of Arsenic in Primary Cultures of Human and Rat Hepatocytes Incubated with 1.0 μM MAsO^{III}

	iAs (pmol/10^6cells)[a]	MAs (pmol/10^6cells)[a]	DMAs (pmol/10^6 cells)[a]	total As (pmol/10^6 cells)[a]
human (donor 2)	37 (32, 42)	662 (658, 666)	1296 (1282, 1310)	1995 (1972, 2018)
rat	32 (27, 36)	172 (147, 196)	2025 (2000, 2049)	2228 (2174, 2281)

[a] Means and values of duplicated samples are shown. Each sample represents combined cell and medium from four wells (10^6 cells and 2 mL of medium). Background amounts of iAs, MAs, and DMAs found in culture media were subtracted from sample values. Detection limits of the HG−AAS procedure are as follows: 7.7 pmol for iAs^{III}, 5.4 pmol for MAs^{V}, and 14.5 pmol for DMAs^{V}. Detection limits were determined as 3σ from five blank samples in which cells and medium were replaced with water.

μM DMAs^{III}GS, or 1 μM DMAs^{V} for 24 h. In both cell types, most of the MAs^{III}O was converted to DMAs (Table 2). DMAs accounted for 65 and 91% of the total arsenic in human and rat hepatocyte cultures, respectively. In contrast, less than 5% of the MAs^{V} was further methylated by either cell type (data not shown). The rate of cellular uptake of MAs^{III} was several-fold greater than that of MAs^{V} (data not shown). DMAs was the major or only metabolite found in rat and human hepatocytes incubated with DMAs^{III}GS or DMAs^{V} (data not shown). The small amount of iAs detected in hepatocytes (Table 2) reflects the normal iAs contents of cells.

Effects of iAs Concentration on the Production of Methylated Metabolites. The concentration dependence of the production of methylated metabolites was examined in human and rat hepatocytes exposed to 0.1−20 μM iAs^{III} for 24 h. Human hepatocytes (donor 3) exposed to 0.1 μM iAs^{III} produced almost equal amounts of MAs and DMAs (Figure 2a). At higher iAs^{III} concentrations, the yield of MAs increased and that of DMAs decreased. Cells retained 72−78% of the MAs that was produced (data not shown). At 10 or 20 μM iAs^{III}, the synthesis of MAs in human hepatocytes was inhibited in a concentration-dependent manner and all MAs was retained in cells. Experiments in which hepatocytes from donors 1 and 4 were used produced similar results (data not shown).

The methylation yield for rat hepatocytes increased between 0.1 and 4 μM iAs^{III}; DMAs was the major metabolite (Figure 2b). Only small amounts of MAs were detected in cells and medium. When the iAs^{III} concentration in culture exceeded 1 μM, the MAs yield increased considerably. At 10 or 20 μM iAs^{III}, DMAs production and the overall methylation yield decreased in a concentration-dependent manner. Rat hepatocytes did not accumulate MAs; at all iAs^{III} concentrations, MAs was mainly found in medium. The viability of either cell type was unaffected by exposure to up to 1 μM iAs^{III} for 24 h. Exposure to 4−20 μM iAs^{III} decreased the viability of human hepatocytes to 70−84% of that found in untreated cells (data not shown). A similar pattern of iAs^{III} cytotoxicity was found in rat hepatocytes (data not shown).

Determination of Methylation Capacities of Human and Rat Hepatocytes. Table 3 shows apparent

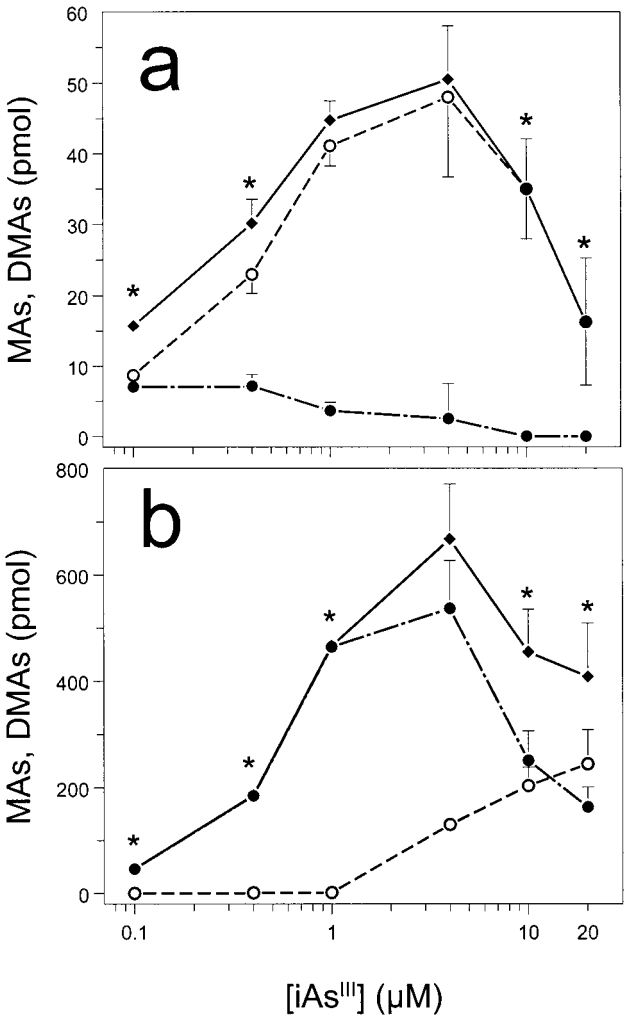

Figure 2. Effects of iAs^{III} concentration on methylation yields in primary cultures of human (a) and rat (b) hepatocytes: MAs (○), DMAs (●), and MAs and DMAs (◆). Human cells (2×10^5) and rat cells (10^5) were plated per well in 0.5 mL of medium. Each point and error bar represents the mean and SD value for four wells. (*) The total methylation yield, MAs and DMAs, is significantly smaller ($p < 0.05$) than the methylation yield at 4 μM iAs as determined by a Student's two-sided *t* test.

methylation rates (AMRs) and DMAs/MAs ratios in human and rat hepatocytes exposed to various concentrations of iAs^{III}. The AMR was calculated as the amount of iAs^{III} converted to MAs and DMAs per hour per 10^6 cells. Exposure to 0.4−4 μM iAs^{III} produced the highest AMR in human hepatocytes. For human hepatocytes obtained from four donors, the maximal AMR ranged from 3.1 to 35.7 pmol of iAs^{III} per 10^6 cells per hour. The DMAs/MAs ratio in whole cultures decreased from 0.7 to 3.3 at 0.1 μM iAs^{III} to 0.01−0.03 at 4 μM iAs^{III}. Medium DMAs/MAs ratios exceeded those in whole cultures but also declined with increasing iAs^{III} concentrations. Similar trends were found for AMR and DMAs/MAs ratios in rat hepatocytes; however, the AMR and DMAs/MAs ratios were substantially higher than those for human cells. The maximal AMR of 387 pmol of iAs^{III} per 10^6 cells per hour was reached in rat hepatocytes exposed to 1 μM iAs^{III}.

Discussion

The liver is commonly held to be the major site for methylation of iAs in humans and other species (*1*). An enzyme that catalyzes mono- and dimethylation of arsenic has been detected and partially characterized in livers from several animal species (*1*). The occurrence of abnormal urinary profiles for methylated metabolites of arsenic in patients with various liver diseases who received small doses of iAs^{III} provides evidence for the central role of the liver in arsenic metabolism in humans (*18*, *19*). However, a recent attempt to detect arsenic methyltransferase activity in a sample of human liver was unsuccessful (*9*). Because tissue was frozen before processing, the absence of enzymatically catalyzed methyltransferase activity may be artifactual (*5*).

The work presented here examined the kinetic and dynamic behavior of arsenicals in primary cultures of normal human hepatocytes obtained from four donors. The metabolic patterns were compared with those found in primary cultures of rat hepatocytes. There were striking differences between the patterns of metabolism in human and rat cells. Compared to rat hepatocytes, human hepatocytes exhibited lower rates for the methylation of iAs and produced relatively more MAs. The higher retention of MAs in human hepatocytes indicates that human cells may contain binding sites with greater affinity for MAs than do rat cells. Interactions of MAs with these binding sites may result in low availability of this metabolite for the second methylation reaction and lower overall methylation rates in human hepatocytes. DMAs was the major excretory product in either human or rat hepatocytes, suggesting that dimethylation of iAs may facilitate its interaction with a membrane efflux pathway. Both rat and human hepatocytes rapidly methylated iAs^{III} and $MAs^{III}O$ to mainly DMAs; however, neither cell type efficiently methylated iAs^{V} or MAs^{V}.

Table 3. Apparent Methylation Rates (AMRs) and DMAs/MAs Ratios in Primary Cultures of Human and Rat Hepatocytes Exposed to 0.1−20 μM iAs^{III}[a]

donor	[iAs^{III}] in culture (μM)	AMR (pmol of iAs^{III} per 10^6 cells per hour)	DMAs/MAs ratio	
			whole culture	medium
human 1	0.1	2.3 ± 0.23	0.7	2.8
	0.4	2.9 ± 0.36	0.2	0.8
	1.0	3.1 ± 0.24	0.03	X[b]
	4.0	2.5 ± 1.08	0.04	X
	10	1.8 ± 1.09	0.01	X
	20	X	X	X
human 2	0.1	5.1 ± 0.19	3.3	9.7
	1.0	35.7 ± 7.7	2.9	NA[c]
human 3	0.1	3.3 ± 0.07	0.7	1.6
	0.4	6.3 ± 0.70	0.3	0.8
	1.0	9.3 ± 0.57	0.1	0.2
	4.0	11.0 ± 2.07	0.1	X
	10	7.3 ± 1.48	X	X
	20	3.4 ± 3.06	X	X
human 4	0.1	3.3 ± 0.28	1.0	2.5
	0.4	7.6 ± 0.78	0.3	0.8
	1.0	5.2 ± 0.44	0.1	0.2
	4.0	4.3 ± 0.89	0.03	X
	10	4.4 ± 3.03	X	X
rat	0.1	71 ± 1.9[d]	211[d]	322[d]
	0.4	264 ± 10.1[d]	73[d]	208[d]
	1.0	387 ± 64.5[d]	12[d]	38[d]
	4.0	278 ± 42.9	1.4	5.1
	10	190 ± 33.5	1.2	1.7
	20	170 ± 42.4	0.3	0.7

[a] AMR (mean ± SD, $n = 4$) and average DMAs/MAs ratios determined for a 24 h incubation period. [b] X means MAs and/or DMAs was not detected in cells and/or medium. [c] NA means not analyzed; arsenic metabolites were analyzed only in combined medium and cells. [d] AMR and DMAs/MAs ratios determined for a 6 h incubation period during which most iAs^{III} was methylated.

Competition among oxyanions for membrane carriers may lower the rate of uptake of pentavalent arsenicals by hepatocytes, limiting their availability for cellular metabolism.

The pattern and extent of arsenic methylation in both human and rat hepatocytes were strongly influenced by the iAs^{III} concentration in the culture. For both species, higher iAs^{III} concentrations reduced the AMR and lowered DMAs/MAs ratios. In particular, the level of production of DMAs by human hepatocytes markedly declined as the iAs^{III} concentration in culture increased. This effect was apparent with noncytotoxic concentrations of iAs^{III} (0.1−1 μM), suggesting that the decline in the level of DMAs production resulted from the inhibition of arsenic methyltransferase by iAs^{III} or MAs that was retained in cells rather than from arsenic cytotoxicity. The inhibitory effect of high iAs^{III} concentrations on methylation reactions, particularly on DMAs synthesis, has been previously described in an in vitro system that contained rat liver cytosol (*4*, *5*). The inhibition patterns found in human hepatocytes indicate that the capacity of human liver to methylate iAs is saturable and could be inhibited by moderate iAs concentrations (0.4−4 μM). Previously reported changes in the DMAs/MAs ratio in urine suggest that the capacity for production of methylated metabolites might be exceeded at a daily intake of 125 μg of iAs^{III} (*20*). In contrast, data from population-based studies indicate that saturation or inhibition of metabolism is unlikely to occur at these levels of iAs exposure (*21*, *22*). However, in the absence of tissue dosimetry data, direct comparisons with the results of this study are difficult.

Maximal AMR values for hepatocytes from four donors vary as much as 10-fold. The exceptionally high AMR for hepatocytes from donor 2, the only female donor, suggests that sex may account for some of the interindividual variation. Interestingly, the ratios of DMAs/MAs (1.6−9.7) found in media of human cells exposed to 0.1 μM iAs^{III} approximated the DMAs/MAs ratios (0.96−10.3) found in urine of human volunteers after ingestion of small doses of iAs^{III} (*23*−*25*). It has been shown in experiments in human volunteers that there is a considerable interindividual variation in the pattern of whole body retention of orally administered iAs (*26*). These interindividual differences could reflect differences in the capacity to methylate arsenic or differences in the retention of one or several of the metabolites in the pathway from iAs to the methylated metabolites. Variations in the capacity to methylate arsenic could arise from differences among individuals in the catalytic activity of the methyltransferase, in the availability of cofactors and methyl group donors, or in the presence of inhibitors of the methylation reactions. The use of primary cultures of human hepatocytes provides data about interindividual variation in the capacity for the methylation of arsenic and in the metabolism and fate of arsenicals in cells. Along with studies of distribution and clearance in intact animals, it should be possible to create a more comprehensive understanding of the metabolism and toxicity of arsenic in humans.

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Supporting Information Available: GSH concentrations, basal and induced P450 (CYP3A4) activities in cultured human hepatocytes, and morphology of the cultured human hepatocytes (donors 2 and 4). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Aposhian, H. V. (1997) Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu. Rev. Pharmacol. Toxicol.* **37**, 397−419.

(2) Cullen, W. R., McBride, B. C., and Reglinski, J. (1984) The reaction of methylarsenicals with thiols: Some biological implications. *J. Inorg. Biochem.* **21**, 179−194.

(3) Yamauchi, H., and Fowler, B. A. (1994) Toxicity and metabolism of inorganic and methylated arsenicals. In *Arsenic in the Environment, Part II: Human Health and Ecosystem Effects* (Nriagu, J. O., Ed.) pp 35−43, Wiley, New York.

(4) Buchet, J. P., and Lauwerys, R. (1985) Study of inorganic arsenic methylation by rat in vitro: relevance for the interpretation of observations in man. *Arch. Toxicol.* **57**, 125−129.

(5) Styblo, M., Delnomdedieu, M., and Thomas, D. J. (1996) Mono- and dimethylation of arsenic in rat liver cytosol in vitro. *Chem.-Biol. Interact.* **99**, 147−164.

(6) Styblo, M., Yamauchi, H., and Thomas, D. J. (1995) Comparative methylation of trivalent and pentavalent arsenicals. *Toxicol. Appl. Pharmacol.* **135**, 172−178.

(7) Lerman, S. A., Clarkson, T. W., and Gerson, R. J. (1983) Arsenic uptake and metabolism by liver cells is dependent on arsenic oxidation state. *Chem.-Biol. Interact.* **45**, 401−406.

(8) Zakharyan, R. A., Wu, Y., Bogdan, G. M., and Aposhian, H. V. (1995) 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.

(9) Zakharyan, R. A., and Aposhian, H. V. (1999) Arsenite methylation by methylvitamin B_{12} and glutathione does not require an enzyme. *Toxicol. Appl. Pharmacol.* **154**, 287−291.

(10) International Agency for Research on Cancer (1987) In *IARC Monograph on the Evaluation of Carcinogenic Risk to Humans: Overall Evaluation of Carcinogenicity: an update of IARC Monographs 1 to 42* (Suppl. 7), p 100, International Agency for Research on Cancer, Lyon.

(11) Styblo, M., Serves, S. V., Cullen, W. R., and Thomas, D. J. (1997) Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chem. Res. Toxicol.* **10**, 27−33.

(12) Reay, P. F., and Asher, C. J. (1977) Preparation and purification of ^{74}As-labeled arsenate and arsenite for use in biological experiments. *Anal. Biochem.* **78**, 557−560.

(13) Strom, S. C., Pisarov, L. A., Dorko, K., Thompson, M. T., Schuetz, J. D., and Schuetz, E. G. (1996) Use of human hepatocytes to study P450 gene induction. *Methods Enzymol.* **272**, 388−401.

(14) Seglen, P. O. (1973) Preparation of rat liver cells. *Methods Cell Biol.* **13**, 29−83.

(15) Styblo, M., Hughes, M. F., and Thomas, D. J. (1996) Liberation and analysis of protein-bound arsenicals. *J. Chromatogr. B: Biomed. Appl.* **677**, 161−166.

(16) Crecelius, E. A., Bloom, N. S., Cowan, C. E., and Jenne, E. A. (1986) Determination of arsenic species in limnological samples by hydride generation atomic absorption spectroscopy. In *Speciation of Selenium and Arsenic in natural Waters and sediments. Volume 2: Arsenic Speciation*, pp 1−28, Electric Power Research Institute, Palo Alto, CA (EA-4641, Project 2020-2).

(17) Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B. (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* **47**, 936−942.

(18) Buchet, J. P., Geubel, A., Pauwels, S., Mahieu, P., and Lauwerys, R. (1984) The influence of liver disease on the methylation of arsenite in humans. *Arch. Toxicol.* **55**, 151−154.

(19) Geubel, A. P., Mairlot, M. C., Buchet, J. P., Dive, C., and Lauwerys, R. (1988) Abnormal methylation capacity in human liver cirrhosis. *Int. J. Clin. Pharm. Res.* **8**, 117−122.
(20) Buchet, J. P., Lauwerys, R., and Roels, H. (1981) Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. *Int. Arch. Occup. Environ. Health* **48**, 111−118.
(21) Hopenhayn-Rich, C., Smith, A. H., and Goeden, H. M. (1993) Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. *Environ. Res.* **60**, 161−177.
(22) Hopenhayn-Rich, C., Biggs, M. L., Smith, A. H., Kalman, D. A., and Moore, L. E. (1996) Methylation study of a population environmentally exposed to arsenic in drinking water. *Environ. Health Perspect.* **104**, 620−628.
(23) Tam, G. K. H., Charbonneau, S. M., Bryce, F., Pomroy, C., and Sandi, E. (1979) Metabolism of inorganic arsenic (^{74}As) in humans following oral ingestion. *Toxicol. Appl. Pharmacol.* **50**, 319−322.
(24) Yamauchi, H., and Yamamura, Y. (1979) Dynamic changes of inorganic arsenic and methylarsenic compounds in human urine after oral intake as arsenic trioxide. *Ind. Health* **17**, 79−83.
(25) Buchet, J. P., Lauwerys, R., and Roels, H. (1981) Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. *Int. Arch. Occup. Environ. Health* **48**, 71−79.
(26) Pomroy, C., Charbonneau, S. M., McCullough, R. S., and Tam, G. K. H. (1980) Human retention studies with ^{74}As. *Toxicol. Appl. Pharmacol.* **53**, 550−556.

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