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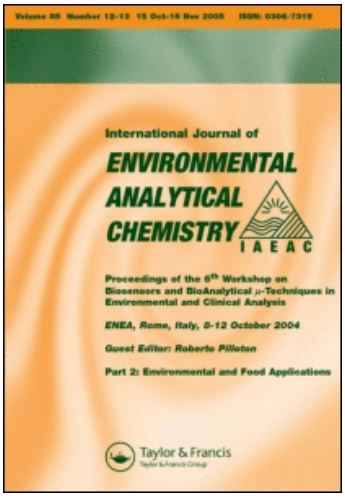

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Significance of Arsenic Metabolic Forms in Urine. Part I: Chemical Speciation

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Significance of Arsenic Metabolic Forms in Urine. Part I: Chemical Speciation†

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The aim of this research has been to develop analytical procedures whereby the various chemical forms of arsenic present in urine can be distinguished and further data on the biotransformation of absorbed arsenic can be acquired.

The separation of inorganic arsenic (InAs), monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA) in urine was performed by ion-exchange chromatography on AG 50 W-X8 resin. Arsenic was then measured directly on the eluted fractions by atomic absorption spectrophotometry, after the reduction of arsenic to the correspondent arsine.

In 160 subjects with no occupational exposure to arsenic compounds, InAs, MMAA, DMAA each accounted for about 10% of the total arsenic urinary excretion (17.2 ± 11.1 μg/l), thus indicating that in the normal population over 60% of arsenic in urine is present in other organic forms. After eating marine food, there was a marked increase of urinary output of arsenic, but no increase was observed in InAs, MMAA and DMAA urinary excretion.

In the biological monitoring of exposure to inorganic arsenic, particularly in the case of high urinary excretion values, the differentiation of the excreted forms of arsenic is necessary to establish with certainty the source (industrial or alimentary) of arsenic.

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KEY WORDS: Inorganic arsenic, biotransformation, blood and urine As concentration.

LIST OF ABBREVIATIONS

InAs	inorganic arsenic
MMAA	monomethylarsonic acid
DMAA	dimethylarsinic acid
MethAs	methylated arsenic (MMAA + DMAA)
InAsMet	inorganic arsenic metabolites (InAs + MethAs)
As^{+3}	arsenic in the oxidation state +3
As^{+5}	arsenic in the oxidation state +5
As_2O_3	arsenic trioxide
AAS	atomic absorption spectroscopy

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INTRODUCTION

Around sixty thousand tons of arsenic were yearly produced in the world from 1967 to 1973. About 50% of the total As production is used in the manufacture of pesticides and wood preservatives, while other minor applications are in the production of metallic arsenic, special alloys, catalysts and pigments, and in the glass industry. Since significant amounts of As are released into the environment by emissions from metal ore smelteries and coal burning systems, arsenic has also gained importance as a general environmental pollutant. Because of its recognized potential as a carcinogenic agent for the respiratory system[2], arsenic exposure may represent a health hazard, especially for people living close to sources of As emissions.

The aims of this work were: (i) to develop an analytical procedure for the determination of total As in blood and urine and for the speciation of As metabolites in urine; (ii) to evaluate blood and urine As concentration in subjects with environmental exposure; (iii) to evaluate the interference of dietary intake of As.

MATERIALS AND METHODS

Standards and Reagents

Standard solutions were prepared with sodium arsenate (C. Erba,

Milan, Italy), monomethylarsonic acid (Alfa Ventron Co., Danvers, USA) and dimethylarsinic acid (Roc/Ric Trimital, Milan, Italy).

Sodium borohydride was obtained from BDH (Poole, England). Buffer solutions were from Merck (Darmstadt, FRG) and the arsenic standard stock solution (1 mg/ml) from BDH. Cationic ion-exchange resin AG 50W–X8, 100–200 mesh was obtained from BioRad (Richmond, USA).

All other reagents were "Analytical Grade" Merck products.

Apparatus

A Perkin–Elmer 5000 atomic absorption spectrometer equipped with a Mercury Hydride System (MHS-1), and an arsenic hollow cathode lamp was used. The spectral line at 193.7 nm with a slit width of 0.7 nm was used. Signals were recorded and measured as peak heights.

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Procedure

Ion-exchange chromatographic separation of metabolic forms of As in urine The procedure adopted was derived from the methods originally described by Tam *et al.*[3,4] Two ml of urine were acidified with 80 μl of concentrated HCl and passed through chromatographic columns filled with 4 g AG 50W-X8 resin (H^+ form). Inorganic Arsenic (InAs), monomethylarsonic acid (MMAA), arsenic in other forms from dietary sources, and dimethylarsinic acid (DMAA) were eluted with (F1) 5 ml of 0.5 N HCl, (F2) 7 ml of H_2O, (F3) 10 ml of 5% NH_4OH (v/v) and (F4) 11 ml of 20% NH_4OH (v/v), respectively.

Mineralization Destruction of the organic matrix is necessary to measure total As content of blood and urine samples. Dry oxidation with $MgO\text{-}Mg(NO_3)_2$,[5] was the method chosen. Blood samples required pretreatment with HNO_3—H_2O_2 before dry-ashing. Minor adjustments were performed in the quantity of added salts (200 mg of MgO and 100 mg of $Mg(NO_3)_2$), and the ashing temperature and time (600°C for 30 min).

RESULTS

Chromatographic separation

Elution patterns of arsenic metabolites are reported in Fig. 1. The overlap between InAs and MMAA is due to contamination of MMAA from InAs. In fact the MMAA from Alfa Ventron was a 95% pure product. Before AAS analysis, pH and volumes of the fractions were modified as follows: to fraction 1 were added 50 μl of $KMnO_4$ solution (6% w/v) and sufficient 0.5 N HCl to give a volume of 10 ml, to fraction 2 were added 0.5 ml of conc. HCl and sufficient H_2O to give a final volume of 10 ml; fraction 4 was dried at 90°C and dissolved in 10 ml 1M acetate buffer pH 4.0. Fraction 3 was usually discarded. When determining As from dietary sources, fractions 3 and 4 were subject to a mineralization procedure (see below) before AAS analysis.

All eluates were diluted to 10 ml because in the MHS-1 system the fractions were analyzed *in toto* and the peak height was dependent

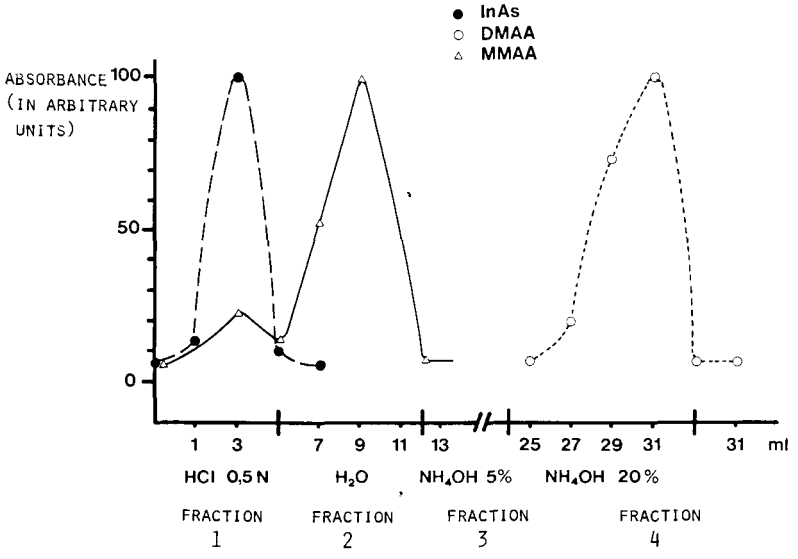

FIGURE 1 Elution patterns of InAs and its methylated derivatives from AG 50W-X8 resin.

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on the volume loaded into the reaction vessel. Since As^{3+} and As^{5+} give different intensities of response, all the arsenic present in fraction 1 must be converted into a single oxidation state before analysis. In spite of the fact that As^{3+} gives a higher signal than does As^{5+}, analysis of InAs in the As^{5+} form was preferred since oxidation with $KMnO_4$ requires only a few seconds, whereas KI reduction requires at least 1 hour. Reduction of fraction 4 was performed in acetate buffer ph 4.0 to obtain the maximum response for DMAA (Fig. 2).

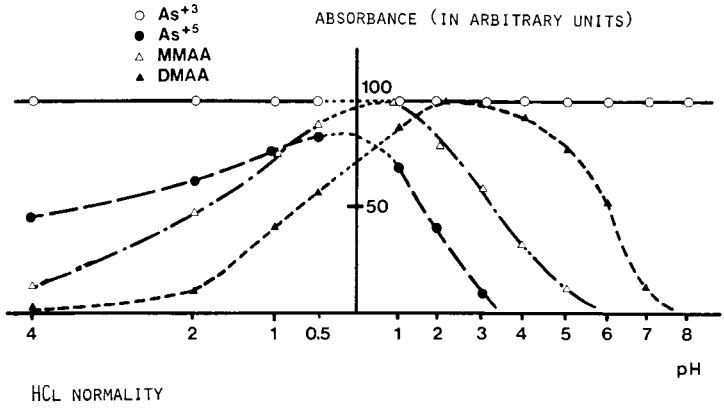

FIGURE 2 pH dependence of arsine production rate.

When measuring arsenic from dietary sources, fractions 3 and 4 were dry-ashed with $MgO\text{-}Mg(NO_3)_2$ at 600°C before analysis.

Ashing of fraction 4 was necessary since a small amount of As from dietary sources elutes together with DMAA and it can only be estimated as a differential measurement of fraction 4 with and without ashing; aqueous solutions of arsenate, MMAA and DMAA were used as AAS standards.

Quality control of the analytical methods

The limit of detection of the forms of As present in urine was 0.5 μg/l for each chemical form. Recovery from 3 urine samples spiked with InAs, MMAA and DMAA averaged 105% for InAs, 98% for MMAA and 104% for DMAA. No significant differences in recovery were

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obtained when analyzing urine containing 200, 400 or 500 μg/l of total As eliminated as organoarsenical compounds after seafood consumption. Precision was assessed by repeated determinations of As metabolite concentration in aliquots of the same samples. The results were as follows: InAs ($n=11$), mean 195 μg/l, CV: 3.6%; MMAA ($n=11$), mean 97.3 μg/l, CV: 3.9% and DMAA ($n=11$), mean 212 μg/l, CV: 5.3%.

The limit of detection of total arsenic in urine or blood samples analyzed after mineralization was 0.5 μg/l. This procedure gave a recovery of 95 to 102% for samples with As concentrations from 40 to 400 μg/l with a CV of 3 to 6%.

Reference values

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Blood and urine As concentrations were measured in samples collected in the morning from 160 apparently healthy males without occupational exposure to As. Their mean age was 33 years (range 20–58). 36% of the subjects were non-smokers, 40% smoked less than a pack of cigarettes per day and 24% smoked a pack or more a day. Sample obtained from subjects who had consumed any kind of marine fish or shellfish within the preceding week were collected as a separate group.

Mean blood As concentration was about one-third of that measured in urine. InAs, MMAA and DMAA each accounted for about 10% of the urinary total-As excretion.

More than 60% urinary As was represented by other forms eluted in the chromatographic fractions 3 and 4 which could have been determined only after dry-ashing (Table I).

Interference of As from diet

The behavior of urinary As excretion after a seafood meal was investigated in 12 reference subjects who reported seafood ingestion in the days preceding sampling. The samples were processed as usual through ion-exchange chromatographic separation, but, in addition, fractions 3 and 4 were also evaluated for total-As content after dry ashing. This allowed us to compare the concentration of urinary As estimated as the difference between total-As and InAsMet and that analytically measured in fractions 3 and 4. The results, shown in Table II, demonstrate a good correspondence between the values obtained by calculation and those by analysis.

TABLE I

Blood and urine As concentration in a reference population of 148 subjects.

	Blood arsenic (μg/l)	Urine arsenic (μg/l)						
		InAs	MMAA	DMAA	MethAs	InAsMet	OF [a]	Total-As [b]
Mean	5.1	1.9	1.9	2.1	4.0	5.9	11.3	17.2
SD	(6.9)	(1.2)	(1.4)	(1.5)	(2.2)	(2.9)	(10.1)	(11.2)
Range	0.5–32	0.5–10	0.5–9	0.5–10	1–14	2–21	0–43	0.5–48

For abbreviations see the list at the beginning of the paper.

[a]Other forms of As, calculated as the difference between total-As and InAsMet.

[b]Determined by ashing of the urine samples *in toto*.

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TABLE II

Blood and urine As concentration of 12 reference subjects who reported seafood consumption in the days preceding the sampling.

Subjects	Blood arsenic (μg/l)	Urine arsenic (μg/l)					
		InAs	MMAA	DMAA	Other[a]	Forms[b]	Total-As
1	4.3	2.0	0.5	1.0	423	440	475
2	7.7	0.5	0.5	1.2	98	90	100
3	15.5	1.6	2.6	2.0	72	82	78
4	39.0	2.8	0.7	0.6	636	555	640
5	5.5	1.0	1.2	1.0	49	66	52
6	62.0	1.6	2.3	2.5	68	80	74
7	26.5	1.8	1.7	3.2	89	95	95
8	14.0	1.7	1.4	1.7	88	102	93
9	0.8	3.1	3.1	1.5	62	58	70
10	1.0	2.3	1.7	2.4	99	76	105
11	9.5	2.9	1.5	1.0	127	142	132
12	5.0	3.5	3.1	3.8	53	60	63
Mean	15.8	2.1	1.7	1.8	126.9	123.7	132.3

[a]Values obtained as arithmetic difference between Total-As and the sum of InAs, MMAA, DMAA.

[b]Values determined after ashing of fractions 3 and 4 eluted from ion-exchange column.

DISCUSSION

The determination of As concentration in the urine has been the dose test most widely used for the biological monitoring of human exposure to As.[2] The studies reported here originated from the experimental observation that urinary excretion of total-As is not a reliable indicator of exposure to inorganic As.[6]

This fact has implications not only for the surveillance of people with occupational or environmental exposure to As, but also for developing a dose-response relationship for As toxicity. In fact, as is probably true for other metals, it is becoming increasingly apparent that the chemical form in which As is present in the organism, as well as its concentration, may be a determinant of toxicity.

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Arsenic is an ubiquitous pollutant of the environment, arsenic intake by the general population occurs through air inhalation as well as water and food consumption. For people who do not have a diet rich in seafood, daily intake of As has been estimated in the order of 10–50 μg As, including both inorganic and organic forms.[2] For people with diets based mainly on seafood, this estimate has to be increased several-fold because seafood is extraordinarily rich in a peculiar form of organic As, recently characterized as an arsenobetaine.[7,8,9]

Limited knowledge is available on the chemical modifications of As in the human organism. Recent studies suggest that inorganic As may be converted to and eliminated in various forms, namely arsenite, arsenate, monomethylarsonic acid, and dimethylarsinic acid.[10,11,12]

According to limited studies,[13,14] As from seafood seems unable to mix with the inorganic As pool in the organism, and thus its toxicity may differ remarkably from that of inorganic As.

The chemical forms of As present in the urine may be easily differentiated and quantitated by ion-exchange chromatographic separation coupled with AAS. The method adopted in this study was developed from those originally proposed by Tam *et al.* [3,4] and Uthe *et al.*,[5] with some modifications. This method allows us to discriminate inorganic arsenic (InAs), monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) in the urine. More over, the ashing procedure adopted to determine total-As concentration in

the samples *in toto* was extended to single chromatographic fractions, allowing an accurate estimate of the other organic forms of As present in the urine. Due to the interference of the organic matrix, this method is not applicable to blood samples, where only the total-As concentration after dry ashing may be measured.

In a reference population without occupational exposure or seafood ingestion, the blood and urinary As measurements show that InAs and its methylated metabolites represent only about 30% of total-As elimination in the urine, the remaining 70% consisting of other organic forms. The values of urinary total-As excretion found in this study are the same order of magnitude as the estimated daily intake of As, thus indicating that under normal conditions a balance occurs between the daily intake of As and its elimination.

Blood As concentration was found to be unrelated to the urinary excretion of total-As. In spite of the fact that the urinary concentration of organic forms of As other than MMAA and DMAA represented more than 60% of the urinary total-As excretion, blood As concentration was significantly correlated only with the urinary excretion of InAs and its methylated metabolites. This suggests that compartmentation of these forms may be entirely different from that of InAs and its derivatives, and that other routes of elimination may take place for such forms.

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Acknowledgement

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