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Biomethylation of Inorganic Arsenic by the Rat and Some Laboratory Animals

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Arsenic is widely distributed in environment, and agricultural use of arsenic pesticides, fungicides and herbicides can produce contamination of the environment with arsenic compounds. And animals including human are exposed to the arsenic through water and food. However, a little information has been published on the changes in the chemical forms of arsenic that occur within the animal body. Recent studies by LAKSO and PEOPLES in cows(1975), by CRECELIUS in human(1977) and by TAM et al. in dogs(1979) have shown that inorganic arsenic is partly metabolized to methylated arsenic. However, these researches were only taken place in a few media such as urine and plasma. Hence, a more detailed behaviour(the dynamics of distribution, and elimination into feces or bile) of arsenic metabolites in the body are not well known.

On the other hand, many researchers reported that marked species difference in the absorption, excretion and tissue binding of arsenic (as total arsenic) exist between the rat and some laboratory animals (DUCOFF et al. 1948; LANZ et al. 1950; PEOPLE 1964; ARIYOSHI and IKEDA 1974; SELBY et al. 1977; FOWLER 1977). However, there have been no reports of species difference in the arsenic methylation.

This report concerns the distribution(in the liver, kidney and blood) and excretion(in the urine, feces and bile) of arsenic metabolites such as dimethylated, monomethylated and inorganic arsenic in rats following a single oral and intravenous(iv) administration of arsenic acid. This paper also describes studies on the species difference in the arsenic methylation between the rats and the other some laboratory animals as mice, hamsters, rabbits and cats.

MATERIALS AND METHODS

APPARATUS. A Hitachi Zeeman atomic absorption spectrometer was used for measurements of all chemical forms of arsenic. The other apparatus used were similar to that of previous publication(1978).

REAGENTS. Reference arsenic compounds were obtained from the following sources : dimethylarsinic acid(cacodylic acid; DMAA), Nakarai Chemical Co.; methanearsonate disodium salts(MAA-2Na), Kumiai Chemical Co.; arsenic acid, Kanto Chemical Co.. The other reagents or chemicals used were similar to those previously described(1978).

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TREATMENT OF ANIMALS. For studies with laboratory animals, male Wistar-Imamichi rats(190±5g), male ICR/JCL mice(36±2g), male Syrian golden hamster(149±5g), male Dutch rabbits(2.0-2.1kg), and male cats(3-4kg) were used. Rats, mice, and hamsters received an oral dose of 5 mg/kg of arsenic acid(0.5 mg As/ml) in water. In another study, arsenic acid of 1 mg/kg(0.5 mg As/ml) in 0.8 % NaCl solution was injected intravenously into rats, mice, hamsters, rabbits and cats. After the treatments, the animals were individually housed in a stainless steel metabolic cages. Urine and feces were collected at daily intervals for 2 days and were kept frozen until analyzed. Food and water were given *ad libitum*. The rats, mice, and hamsters were anesthetized with nembutal, and blood specimans were obtained by inferior venipuncture 2 days after the treatments. Liver and kidney were also taken from these animals at sacrifice and frozen until analyzed. Additional male rats with surgically implanted and externalized biliary catheters received arsenic acid by the oral(5 mg/kg) or iv(1 mg/kg) route, and then bile was collected for 0-6 and/or 6-24 h.

EXTRACTION, ISOLATION, IDENTIFICATION AND MEASUREMENTS OF ARSENIC. Analytical technique used to extract, isolate and identify arsenic metabolites from biological samples was previously described (1978). Arsenicals in materials were extracted with NaOH solution and the extractants were separated by benzene extraction and back extraction with water. The water layers were combined, condensed, and spotted on thin-layer plate coated with cellulose powder. The cellulose layer of the developed plate representing the fractions [dimethylated(DM-As), monomethylated(MM-As) and inorganic arsenic (Inorg-As)] were scraped and extracted with water. The determination of arsenic contents of the each solution was performed utilizing atomic absorption spectrometry similar to that of previous report (1979). Recovery of DMAA, MAA-2Na and arsenic acid added to control samples were almost quantitative in this study.

RESULTS

The excretion of arsenic metabolites in urine and feces, as percentage of dose, following a single oral and iv administration of arsenic acid to the rats, mice, hamsters, rabbits and cats is shown in Table 1 and 2.

Urinary excretion of total arsenic accounted for 17.2, 48.5 and 43.8 %, and fecal elimination for 33.0 48.8 and 44.1 % of the oral administered dose within 48 h in the rats, mice and hamsters, respectively(Table 1). In the mice and hamsters, about 90-100 % of the injected dose were recovered in the urine and feces, whereas in the rats, only about 50 % was excreted. A similar difference of the recovery of total arsenic in excreta was observed in the three animal species following iv administration. The rats, mice and hamsters excreted 51.8, 89.5 and 87.9 % of the iv administered dose, respectively, in the urine and feces during 48 h(Table 2).

In the rats after the oral treatment, a major urinary metabolite of the arsenic(14.1 % of the dose) was excreted as inorganic arsenic.

TABLE 1

Urinary and Fecal Metabolites Obtained from Three Species of Animals after Oral Administration

Animal tested	Speciman	Hours postdose	% of dose recovered DM-As	MM-As	Inorg-As	Total-As
Rat	Urine	0-24	1.8±0.2	0.8±0.2	13.8±1.2	16.4
		24-48	0.4±0.08	0.1±0.02	0.3±0.08	0.8
		Total	2.2	0.9	14.1	17.2
			(13)	(5)	(82)	(100)
	Feces	0-24	0.4	5.2	19.8	25.4
		24-48	0.7	2.5	4.4	7.6
		Total	1.1	7.7	24.2	33.0
			(3)	(23)	(73)	(100)
	Total		3.3	8.6	38.3	50.2
Mouse	Urine	0-24	21.3±0.5	1.2±0.3	16.2±1.6	38.7
		24-48	8.7±3.3	0.6±0.2	0.5±0.2	9.8
		Total	30.0	1.8	16.7	48.5
			(62)	(4)	(34)	(100)
	Feces	0-24	7.5	6.4	18.6	32.5
		24-48	6.9	3.0	6.4	16.3
		Total	14.4	9.4	25.0	48.8
			(30)	(19)	(51)	(100)
	Total		44.4	11.2	41.7	97.3
Hamster	Urine	0-24	9.5±2.3	2.2±0.1	16.7±2.3	28.4
		24-48	12.0±0.5	2.4±1.2	1.0±0.3	15.4
		Total	21.5	4.6	17.7	43.8
			(49)	(11)	(40)	(100)
	Feces	0-24	<0.1	0.7	5.3	6.0
		24-48	1.4	13.2	23.5	38.1
		Total	1.4	13.9	28.8	44.1
			(3)	(32)	(65)	(100)
	Total		22.9	18.5	46.5	87.9

These data represent the values excluding the control values.
Urine : average of four animals±SD.
Feces : average of two animals.
( ) : percentage of each arsenic metabolite to the total arsenic amounts.

The methylated arsenic was also eliminated in the dimethylated form (2.2 %) and the monomethylated form(0.9 %) as a minor metabolite. Most of the arsenic in the feces was inorganic arsenic that represented 24.2 % of the dose, whereas a relatively large portion of monomethylated arsenic(7.7 %) were also present. In iv study, the pattern of urinary excretion of the three arsenic metabolites was similar to that observed in oral administration except for

TABLE 2

Recovery of Metabolites in Urine and Feces following IV Administration of Arsenic Acid to Five Animal Species.

Animal tested	Speciman	Hours postdose	% of dose recovered			
			DM-As	MM-As	Inorg-As	Total-As
Rat	Urine	0-24	2.5±1.1	0.7±0.2	47.2±8.7	50.4
		24-48	0.2±0.07	<0.1	0.4±0.03	0.6
		Total	2.7	0.7	47.6	51.0
			(5)	(1)	(93)	(100)
	Feces	0-24	0.1	0.3	0.3	0.7
		24-48	<0.1	<0.1	0.1	0.1
		Total	0.1	0.3	0.4	0.8
			(12)	(37)	(50)	(100)
	Total		2.8	1.0	48.0	51.8
Mouse	Urine	0-24	34.4±0.04	1.8±0.7	46.2±5.0	82.4
		24-48	3.0±1.0	0.3±0.04	1.2±0.4	4.5
		Total	37.4	2.1	47.4	86.9
			(43)	(2)	(55)	(100)
	Feces	0-24	0.8	0.2	0.6	1.6
		24-48	0.2	0.2	0.6	1.0
		Total	1.0	0.4	1.2	2.6
			(38)	(15)	(46)	(100)
	Total		38.4	2.5	48.6	89.5
Hamster	Urine	0-24	35.5±2.0	1.5±0.3	40.8±3.9	77.8
		24-48	4.2±0.8	0.3±0.05	1.6±0.3	6.1
		Total	39.7	1.8	42.4	83.9
			(47)	(2)	(51)	(100)
	Feces	0-24	0.6	1.7	0.6	2.9
		24-48	0.2	0.5	0.4	1.1
		Total	0.8	2.2	1.0	4.0
			(20)	(55)	(25)	(100)
	Total		40.5	4.0	43.4	87.9
Rabbit	Urine	0-48	25.7	2.3	49.5	77.5
			(33)	(3)	(64)	(100)
Cat	Urine	0-48	29.4	1.0	31.5	61.9
			(48)	(2)	(51)	(100)

These data represent the values excluding the control values.
Urine of rats, mice and hamsters : average of four animals±SD.
Feces of rats, mice and hamsters, and urine of rabbits and cats : average of two animals.

a difference in quantity of inorganic arsenic. Feces contained dimethylated, monomethylated and inorganic arsenic which represented less than 1 % of the dose.

In the mice given the oral administration, the urinary excretion ratios of monomethylated(1.8 %) and inorganic arsenic(16.7 %) were similar to that found in the rats. But the ratio of dimethylated arsenic was quite different, inasmuch which was excreted as main metabolite(30.0 % of the dose). A similar phenomenon was also observed in feces. The fecal elimination of monomethylated(9.4 %) and inorganic arsenic(25.0 %) were similar with those in the rats, whereas the amount of dimethylated arsenic(14.4 %) was quite unlike. In iv administration, the content of dimethylated arsenic(37.4 %) in the urine was also much greater than seen in the rats.

In the hamsters treated orally, the dimethylated arsenic(21.5 % of the dose) was found as major metabolite in the urine in the like manner as the mice. The excretion ratio of inorganic arsenic(17.7 %) was similar to that found in the rats and mice. The amount of monomethylated arsenic(4.6 %) was highest in the three animal species. And that was also observed in the feces. The values of inorganic arsenic(28.8 %) in the feces was similar in excretion ratios to the rats and mice, whereas the amount of dimethylated arsenic(1.4 %) was similar with those in the rats but differ from those in the mice. In iv treatment, dimethylated(39.7 %) and inorganic arsenic(42.4 %) as major metabolites, and monomethylated arsenic(1.8 %) as minor were excreted in the urine in the similar way as the mice.

There was not much difference in the pattern or in the ratio of the fecal excretion of the three arsenic metabolites among the rats, mice and hamsters after iv administration, since only small amounts(0-2 % of the dose) of each arsenic metabolite was detected for 2 days after the dosing.

Major urinary metabolites of the arsenic in the rabbits and cats following iv administration were excreted as dimethylated(25.7 and 29.4 %, respectively) and inorganic arsenic(49.5 and 31.5 %, respectively), and this observation was not very different from that of the mice and hamsters.

The tissue distribution of arsenic metabolites in the rats, mice and hamsters 2 days after the treatment is presented in Table 3. With oral administration, the total arsenic levels of whole blood in the rats were extremely higher than those in the mice and hamsters. Approximately 44 % of the arsenic dose remained in the rat blood, and over 98 % of the residual arsenic was dimethylated form of arsenic. The liver and kidney of the rats also contained the dimethylated arsenic as the main metabolite. A similar result was also observed in the rats following iv administration. On the other hand, most of the residual arsenic in the tissue of the mice and hamster after oral and iv administration were too low to be identified in this study.

The biliary excretion of arsenic metabolites in the rats is presented in Table 4. In oral study, monomethylated arsenic was found as a major metabolite of arsenic in bile and accounted for 4-5 % of the administered dose within 24 h. The dimethylated and inorganic arsenic were also found, but in lower concentration.

TABLE 3

Tissue Distribution of Metabolites in Rat, and Total Arsenic in Mouse and Hamster after Oral and IV Administration.

% of dose		Rat				Mouse	Hamster
Route of admin.	Tissue	DM-As	MM-As	Inorg-As	Total	Total	Total
Oral	Blood*)	43.6 (98.3)	0.6 (1.4)	0.1 (0.2)	44.3 (100.0)	0.1	<0.1
	Liver	2.0	0.1	0.2	2.3	0.2	0.2
	Kidney	0.4	0.1	0.1	0.6	0.1	0.5
IV	Blood*)	37.6 (94.3)	1.3 (3.2)	1.0 (2.5)	39.9 (100.0)	<0.2	0.1
	Liver	1.5	0.1	0.1	1.7	0.3	0.1
	Kidney	0.4	0.1	0.2	0.7	0.3	0.1

Average of two animals. ( ) : percentage of each arsenic metabolite to the total arsenic amounts.
*)These data in the blood were obtained using the formular : [concentration of arsenic in the treated blood(ppm)- concentration of arsenic in the control blood(ppm)]× body weight(g)× 0.07× 100÷ total arsenic dose(μg). The other data represent the values excluding the control values.

TABLE 4

Biliary Metabolites Obtained from Rat after Oral and IV Administration of Arsenic Acid.

Route of admin.	Hours postdose	% of dose recovered DM-As	MM-As	Inorg-As	Total-As
Oral	0-6	0.1±0.02	2.4±1.0	0.1±0.1	2.6
	6-24	0.1±0.02	1.8±0.6	0.1±0.07	2.0
	Total	0.2	4.2	0.2	4.6
IV	0-6	<0.1	0.2±0.04	<0.1	0.2
	6-24	<0.1	<0.1	<0.1	<0.1
	Total	<0.1	0.2	<0.1	0.2

Average of four animals±SD.
These data represent the values excluding the control values.

The biliary excretion pattern of the three arsenic metabolites after iv administration were similar to that after oral administration excepting difference in quantity.

DISCUSSION

In the rats studied, about half of the orally administered dose of the arsenic was excreted in the urine and feces(bile), and most of the remainder was found in the blood. The inorganic arsenic in the urine and feces, monomethylated arsenic in the bile, and dimethylated arsenic in the blood were detected as predominant metabolites, respectively. It seemed that most of the inorganic arsenic found in the feces was not absorbed from the gastrointestinal tracts, since the amount of inorganic arsenic in the bile was extremely small. According to the similarity on the content of monomethylated arsenic between the biliary and fecal excretion, the monomethylated arsenic detected in the feces may be derived from the bile, and may not be methylated in the intestinal tracts by bacterial flora. The presence of a great deal of dimethylated arsenic in the blood and low enterohepatic recirculation of methyl-As after iv administration may suggest that methylation of arsenic could occur in the rat body except in the intestinal microorganisms.

In the three species studied, the rats, mice and hamsters, notable differences in the fecal arsenic metabolites in oral administration were evident. The elimination ratio of dimethylated arsenic in the mice was extremely higher than those of the rats and hamsters, and monomethylated arsenic in the hamsters was comparatively higher than those of the rats and mice, despite of similarity on the inorganic arsenic in the three animal species. On the supposition of the little occurence of arsenic methylation by intestinal microorganisms, these differences might be due to the differences on the biliary secretion among the three animal species.

Furthermore, a marked difference in quantity of the urinary excretion of arsenic metabolites was observed in the three animal species(the rats, mice and hamsters) after oral administration. Excretion ratio of dimethylated arsenic in the rats(2-3 % of the dose) are remarkably less than seen in the mice and hamsters(20-30 %). A quite similar difference was also observed in the urinary excretion of the rats, mice and hamsters following iv administration. In addition to this, the urinary excretion ratios of the arsenic metabolites in the rabbits and cats after iv administration were similar to those in the mice and hamsters. Therefore, the rat is distinguished from the mouse, hamster, rabbit and cat by low excretion of dimethylated arsenic in the urine.

It is well known that arsenic has a peculiar high affinity for the red blood cells and its half life in the blood is extremely long in rat. Our investigation has made it clear that dimethylated arsenic was the predominant metabolite in the rat blood and its excretion into urine was very low compared with those of the other animal species. From these aspects, it is possible that the dimethylated arsenic may appear to be an essential component for the high affinity to rat-erythrocyte.

Thus, the excretion and distribution of arsenic metabolites (especially in dimethylated arsenic) in the rat differ from those in the other animal species. This divergence is noteworthy since the rat has been used frequently in pharmacologic and toxicologic studies.

Methylation of arsenic has been generally known in micro-organisms. However, there are a few reports on the methylation of arsenic in mammals. LAKSO and PEOPLES(1975) studied the amount of MA(as a mixture of DMAA and MAA) in the urine following administration of arsenate and arsenite to the cows and dogs. CRECELIUS(1977) found DMAA and MAA in human urine after drinking arsenite-rich wine. TAM et al.(1979) examined the urine and plasma of the dogs treated with arsenic acid and found dimethylated and monomethylated arsenic. Our investigation showed that methylation of inorganic arsenic (arsenate) to monomethylated and dimethylated arsenic could occur in animal species such as the rat, mouse, hamster, rabbit and cat. Therefore, it is conceivable that the biological methylation of arsenic is a general phenomenon in mammals.

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