ENVIRONMENTAL RESEARCH 40, 380–390 (1986)

Concentration and Chemical Status of Arsenic in the Blood of Pregnant Hamsters during Critical Embryogenesis

2. Acute Exposure

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Received February 10, 1985

We have determined the concentration and chemical composition of arsenic in the blood of pregnant hamsters between 0.2 and 6 hr after an intraperitoneal injection of a teratogenic dose of radiolabeled sodium arsenate on the morning of the eighth day of gestation. Arsenic was present in plasma and red cells 0.20 hr postinjection. The plasma arsenic concentration reached a maximum of 220 μmole/kg blood near 0.5 hr postinjection. Plasma arsenic existed entirely as low-molecular-weight species. Both arsenite and dimethylarsinate (DMA) were present in plasma 0.20 hr postinjection, indicating that arsenate reduction and methylation of arsenic are rapidly initiated. However, the arsenite contribution remained small while the DMA contribution increased with time. Red cell arsenic included macromolecular arsenic (AsP) as well as three low-molecular-weight forms. The contribution of DMA remained small, but arsenite and AsP contributions increased with time. These findings identify the maternal blood concentration and chemical status of arsenic following the administration of a teratogenic dose of arsenate during the period of organogenesis. They could prove useful for predicting the likelihood of a teratogenic outcome in other mammalian species.

INTRODUCTION

Arsenic is a potent teratogen for mammalian species. In the hamster, acute (Ferm and Carpenter, 1968) or chronic (Ferm and Hanlon, 1986) administration of arsenic in the form of arsenate produces the same spectrum of fetal abnormalities, predominately neural tube defects. These findings strongly infer that both protocols produce the requisite concentration and chemical status of arsenic at vulnerable sites within the maternoembryonic unit of the hamster during organogenesis. The location of these sites has not been identified, although the placenta and early embryo are likely candidates (Hanlon and Ferm, 1977; Morrissey and Mottet, 1983; Lindgren *et al.*, 1984). Since the maternal circulatory system is the agent of delivery the chemical status of arsenic in maternal plasma and red cells should dictate the concentration and chemical composition of arsenic at the critical sites during the period of sensitivity, Day 8 in the hamster (Ferm, 1965).

In this report we offer our findings of the concentration, availability, and chemical composition of arsenic in the plasma and the red cells of hamster dams following the intraperitoneal injection of a single teratogenic dose of arsenate on the morning of the eighth day of gestation. The information obtained identifies conditions in the blood chemistry of arsenic associated with the production of fetal abnormalities in the hamster following acute dosing with arsenate. These findings

0013-9351/86 $3.00

may prove valuable for predicting the teratogenic threat to humans of acute exposure to arsenic during early pregnancy.

MATERIALS AND METHODS

1. Reagents

All reagents used were of analytical grade. Disodium arsenate was radiolabeled with carrier-free arsenic-74 obtained from Amersham. A significant portion of this material is actually acid in newly obtained material. However, dilution with nonradiolabeled arsenate quickly converts the trivalent arsenic to its pentavalent form. A determination of the oxidation state was made on arsenate solutions immediately prior to injection using the ion-exchange analysis described below. All arsenic-74 was present as arsenate.

Dowex 2 × 8 (100 mesh) was purchased in the chloride ion form and Bio–Rad AG-50 × 8 (100 mesh) in the sodium ion form. Both resins were treated according to recommended procedures prior to use.

2. Dosing and Sampling Protocols

Pregnant Syrian hamsters (LAK:LVG) were given an acute (bolus) intraperitoneal injection of radiolabeled sodium arsenate (64.2 μmole/kg dam) at 8:00 AM on the eighth day of gestation. The volume of the injected solution was standardized at 0.5 ml per 100 g of maternal body wt. Individuals were sacrificed at specific times, from 0.20 to 6 hr postinjection.

Maternal blood was separated into plasma and red cell fractions by centrifugation at 1800*g*. Total arsenic concentrations were obtained by radioassay as previously described (Hanlon and Ferm, 1986).

3. Availability of Arsenic in Maternal Blood

Plasma and red cells were obtained as described above. Treatment of plasma and red cell samples, gel filtration, and dialysis experiments were performed following methods detailed in a previous report (Hanlon and Ferm, 1986). An ethanol precipitation method was also used to determine the availability of arsenic in red cells. This method is described below (Sect. 4).

4. Quantitative Analysis of Arsenic Metabolites

Identification and quantitation of arsenate and its metabolites were achieved using ion-exchange chromotography. Somewhat different methods were utilized for plasma and red cell sap samples.

For plasma samples, which were found to contain only nonprotein bound arsenic (see Results), arsenate was separated from other arsenic forms by chromatography on Dowex 2 × 8 (100 mesh). Assays were made on 1 to 3 ml of plasma or concentrated red cell sap dialysate. Isolab Practi-columns (250 × 8 mm, i.d.) were filled with 10 cm^3 of resin (Cl form) suspended in 10 mM Tris (C1), pH 8.0. Fifteen milliliters of 10 mM Tris (C1), pH 8.0, was run through the column and collected as 3-ml fractions. The resin was expelled from the column into glass

sample tubes by light positive pressure and assayed for radioactivity along with the Tris buffer-eluting fractions. Under these conditions arsenate remains tightly bound to the Dowex-2 resin while arsenite and methylated forms of arsenic emerge in the Tris buffer-eluting fractions. Arsenate can be removed from Dowex-2 elution with 4 M HCl, but radioassay of the resin itself proved simpler. There was no decrease in detectable arsenic-74 counts due to absorption by the resin matrix. Separation of arsenite and the methylated arsenic species was achieved using a modification of the AG-50 method of Tam *et al.* (1978) described previously (Hanlon and Ferm, 1986).

Arsenic metabolites recovered in the concentrated dialysate samples were resolved as inorganic arsenic (As_i) and the methylated species using the AG-50 method. Separation of the two inorganic species, arsenate and arsenite, on Dowex-2 was obviated by the high salt concentration in the concentrated dialysates.

Red cell arsenic tightly bound to protein (AsP) and available low-molecular-weight metabolites of arsenic were separated and the arsenic species characterized as follows. Red cells were lysed, centrifuged, and diluted with Tris buffer as described above (Sect. 3). Sample volumes were adjusted to 15 ml with Tris buffer and transferred to 25 ml-screwcapped vials. Six cubic centimeters of dry Dowex-2 resin was added to the vial contents and the mixture shaken for 6 hr at room temperature using a Thomas rotating apparatus set at a speed of 7.5. Liquid resin were separated by pipet. The resin was washed twice with 5 ml of Tris buffer. Washings were combined with the liquid fraction. Resin and liquid were radioassayed as described above. Counts bound to the resin represent the arsenate content. The liquid, containing arsenite, methylated arsenic, and arsenic tightly bound to protein, was acidified to near pH 4.0 by adding 0.05 vol of 1 M NaH_2PO_4. A volume of ethanol equal to the liquid volume was added slowly with stirring and the mixture allowed to stand at room temperature for 15 min. Precipitated material and supernatant were separated by centrifugation at 1800*g* for 5 min. The precipitate was washed with 5 ml of 50% aqueous ethanol containing 0.05 M NaH_2PO_4 and recentrifuged. The wash was combined with the ethanol supernatant. Supernatant, containing arsenite and methylated arsenic, and precipitate, containing arsenic tightly bound to protein, were radioassayed as above. The volume of supernatant was reduced to 3 ml under reduced pressure using a rotary evaporator. Slight heat was applied to the concentration flask to enhance the rate of evaporation. Arsenite and methylated arsenic species in the concentrated supernatant were separated and quantified by chromotography on AG-50 resin.

RESULTS

1. Arsenic Concentrations in Whole Blood, Plasma, and Red Cells

Table 1 shows arsenic concentrations in whole blood, plasma, and red cells for samples collected at different times postinjection. Arsenic, injected intraperitoneally as arsenate, was present in the maternal blood 12 min (0.20 hr) postadministration. Its concentration increased to a maximum near 0.5 hr, then fell rapidly

TABLE 1

THE CONCENTRATION OF ARSENIC IN MATERNAL WHOLE BLOOD, PLASMA, AND RED CELLS AT DIFFERENT TIMES FOLLOWING A SINGLE INTRAPERITONEAL INJECTION OF ARSENATE 8 AM ON THE EIGHTH DAY OF GESTATION

Time postinjection (hr)	No. of samples	μmole arsenic/kg		
		Whole blood	Plasma	Red cells
0.20	1	42.9	66.4	14.3
0.25	4	73.4 ± 4.2	105 ± 7	34.3 ± 1.5
0.50	1	218	315	100
0.53	1	222	358	99.8
0.63	1	149	212	72.1
1.0	4	38.4 ± 1.1	46.2 ± 1.1	29.6 ± 2.0
2.0	4	21.9 ± 0.9	17.7 ± 1.2	24.2 ± 2.6
3.0	3	11.0 ± 2.7	10.2 ± 2.0	11.9 ± 3.7
4.0	4	9.22 ± 1.31	7.23 ± 1.98	11.5 ± 1.68
5.0	3	6.01 ± 0.61	5.69 ± 0.66	6.45 ± 0.75
6.0	3	6.65 ± 1.68	5.88 ± 2.24	7.56 ± 1.19

Note. The dose level was 64.2 μmole/kg dam. Values represent mean ± standard errors.

to 1.0 hr postinjection. Thereafter the rate of decrease was substantially reduced (Fig. 1).

The pattern of rise and fall for whole blood was shared by plasma and red cells. Both blood compartments contained arsenic 0.20 hr postinjection and manifested an arsenic concentration peak near 0.5 hr postinjection. However, the plasma

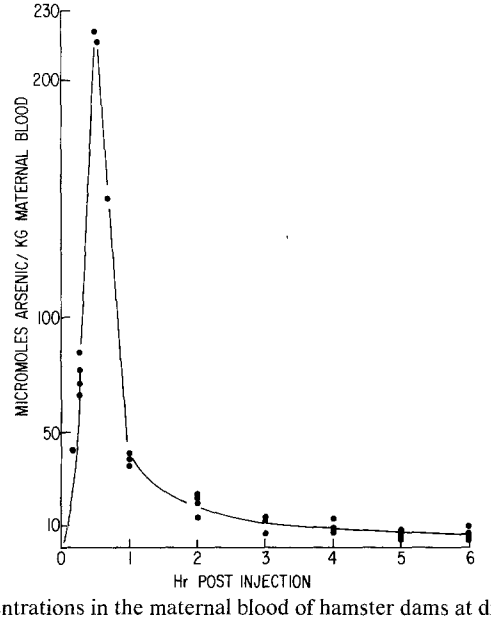

FIG. 1. Arsenic concentrations in the maternal blood of hamster dams at different times following a single teratogenic injection of arsenate (64.2 μmol/kg dam) on the eighth day of gestation. Data points represent individual values used to construct Table 1. A "best-fit" line has been superimposed on the data points.

arsenic concentration was 4.6× that of red cells at 0.20 hr postinjection and 3.2× that of red cells at 0.5 hr postinjection. Three hours after injection, plasma and red cell arsenic concentrations were nearly identical and remained so to 6 hr postinjection.

2. *The Availability of Arsenic in the Plasma and Red Cell Compartments of Maternal Blood.*

Plasma arsenic. Radiolabeled arsenic eluted as a single peak at 1.0 included volumes when samples were chromotographed on Sephadex G-25. A few plasma samples were examined by means of dialysis and ethanol precipitation methods. Plasma samples collected 1 and 6 hr postinjection had the same $t_{1/2}$ value (1.0 hr) on dialysis. No plasma arsenic remained undialyzed. All of the arsenic counts were found in the ethanol supernatant fractions of samples collected 1 and 6 hr postinjection.

Red cell arsenic. Radioassay of fractured red cell membranes showed no arsenic. Red cell arsenic was associated entirely with the soluble fraction. Gel filtration chromotography of red cell sap using Sephadex G-25 showed a poorly resolved arsenic peak near 1.0 vol that was skewed toward the excluded volume in samples collected 1, 3, 4, and 6 hr postinjection.

Dialysis runs were made in triplicate on red cell sap samples from animals sacrificed 1, 3, 4, and 6 hr postinjection. The data presented in Table 2 reveal a time-dependent increase in the amount of red cell sap arsenic which is tightly bound to macromolecules. A measure of the rate of dialysis was obtained by determining $t_{1/2}$, defined as the time required to remove one-half of the dialyzable arsenic. The $t_{1/2}$ for 1-hr samples averaged 1 hr. The $t_{1/2}$ values for 3- and 6-hr samples ranged from 1.5 to 2.0 hr.

Data from the ethanol precipitation method also appear in Table 2. Some arsenic tightly bound to protein (AsP) was present in the samples collected at 0.25

TABLE 2
THE ARSENIC METABOLITES IN MATERNAL PLASMA AT DIFFERENT TIMES FOLLOWING A SINGLE INTRAPERITONEAL INJECTION OF ARSENATE 8 AM ON THE EIGHTH DAY OF GESTATION

Time postinjection (hr)	No. of samples	Total arsenic concentration (μmoles/kg)	Percentage of total arsenic concentration		
			As(V)	As(III)	DMA
0.20	1	66.4	98	0	2
0.25	4	105 ± 7	99 ± 1	0	1 ± 1
0.50	1	315	100	0	0
0.53	1	358	100	0	0
0.63	1	212	99.1	0.4	0.5
1.0	4	46.2 ± 1.1	93 ± 2	2	5 ± 2
2.0	2	17.7 ± 1.2	84	0	16
3.0	3	10.2 ± 2.0	73 ± 3	2 ± 1	28 ± 2
4.0	4	7.23 ± 1.98	57 ± 7	8 ± 6	35 ± 10
5.0	3	5.69 ± 0.66	36 ± 7	15 ± 4	53 ± 7
6.0	3	5.88 ± 2.24	44 ± 7	7 ± 4	49 ± 5

Note. The dose level was 64.2 μmole/kg dam. Values represent mean ± standard deviation.

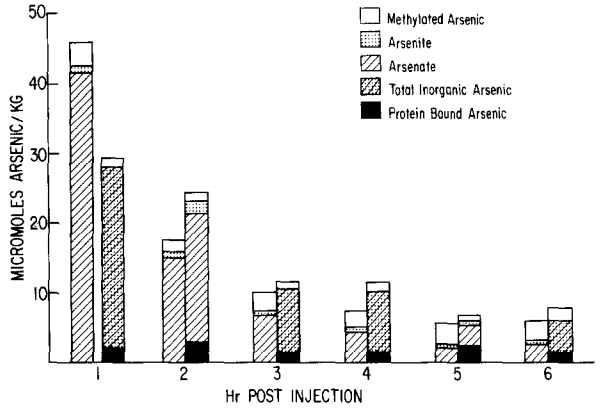

FIG. 2. The chemical composition of arsenic in plasma and red cell sap at different times following a single teratogenic injection of arsenate (64.2 μmole/kg dam) on the eighth day of gestation. The left hand member of each bar group represents findings for plasma, the right hand member represents data for red cell sap. Inorganic arsenic (As_i) represents arsenate and arsenite combined.

hr postinjection. As with the dialysis findings, the ethanol precipitation method showed that AsP contributed increasingly to the total arsenic concentration in red cell sap as the time postinjection increased. However, values for AsP were consistently higher in the ethanol precipitation method.

3. Arsenic Metabolites in Plasma and Red Cell Sap

Three low-molecular-weight species of arsenic were found in maternal blood plasma and red cell sap following an acute injection of arsenate at 64.2 μmole/kg dam. Inorganic arsenic (As_i) was represented by arsenate and arsenite. Organic arsenic was represented by dimethylarsinate (DMA). The same low-molecular-weight species of arsenic were found in red cell sap along with arsenic tightly bound to macromolecules. The contributions of the four forms of arsenic to the total concentrations in plasma and red cell sap are presented in Table 2 and in Fig. 2 for the interval from 1 to 6 hr postinjection.

DISCUSSION

1. Arsenic Concentration in Maternal Blood

A single intraperitoneal injection of a teratogenic dose of arsenate achieves a rapid uptake of arsenic by the maternal blood circulatory system (see Table 1, Fig. 1). The maximum arsenic concentration of 220 μmole/kg, obtained near 0.5 hr postinjection, accounts for 14% of the dose administered (assuming that the maternal blood contributes 6% of the total weight of the hamster). By 5 hr postinjection the maternal blood arsenic concentration decreased by 37× to 6 μmole/kg. The slow rate of decline in blood arsenic concentration observed for the 1- to 6-hr interval apparently continues throughout the first day postinjection. Twenty-four hours after an intravenous injection of the same dose of arsenate given in the present study the maternal blood concentration of arsenic was 3.5 μmole/kg in hamsters dosed on the eighth day of gestation (Hanlon and Ferm, 1977).

The pattern of blood clearance seen here in hamsters has also been reported for mice (Vahter, 1981) and humans (Bertolero *et al.*, 1981) following acute administrations of arsenate at much lower dose levels. This pattern is consistent with the existence of a biphasic kinetic mode of arsenic excretion. Indeed, acutely administered arsenic is rapidly excreted from several mammalian species, but at rates too slow to account for the kinetics of arsenic loss from maternal blood (Pomeroy *et al.*, 1980). More likely, rapid uptake of arsenic by various tissues accounts for the pattern of loss of blood arsenic following acute administration of arsenate. Vahter (1983) cites several studies on rabbits and mice which have shown that single doses of inorganic arsenic produce arsenic concentrations above that of the blood in liver, the kidney, and other tissues. We also found that arsenic was rapidly taken up by the hamster liver, kidney, and placenta and that the pattern of rise and fall in the total arsenic concentration in these tissues was quantitatively similar to that of maternal blood (D. P. Hanlon and V. H. Ferm, unpublished data).

Arsenic, administered intraperitoneally as arsenate, was found in plasma and red cells at all sampling times postinjection (see Table 1). However, the rise and fall of red cell arsenic concentrations lagged behind those of the plasma and at no time during the first hr postinjection exceeded it. At 2 hr postinjection the average red cell concentration of arsenic was greater than that of plasma and remained so to 6 hr postinjection, although not at the level of statistical significance. Vahter and Norin (1980) also found the red cell/plasma ratio near 1.0 for the interval of 2 to 24 hr postdosing in mice receiving an oral dose of 12.8 μmole arsenate/kg body wt. Apparently, the hamster red cell membrane, while permeable to arsenic, provides a degree of resistance to arsenic transport.

2. The Availability of Arsenic in Maternal Plasma and Red Cells

Gel filtration of plasma revealed no protein bound arsenic in samples collected to 6 hr postinjection. The possibility of some weak electrostatic interactions between low-molecular-weight arsenic species and plasma proteins prior to dilution with buffer cannot be discounted, but, in any case, would not contribute importantly to the chemistry of plasma arsenic. Exhaustive dialysis or ethanol precipitation of plasma samples showed no arsenic tightly bound to protein.

All of the red cell arsenic was found in the red cell sap (cytosol). Unlike plasma arsenic, a portion of the red cell arsenic was bound to protein at all sample times postinjection. Gel filtration, dialysis, and ethanol precipitation studies collectively demonstrated that some of the arsenic associated with a protein component (presumably hemoglobin) is weakly bound and some is very tightly bound. The weakly bound component was revealed by the elution profiles in gel filtration runs of red cell sap samples collected 1, 3, and 6 hr postinjection. The dialysis method is less able to detect weakly bound or rapidly exchanged ligands and could not distinguish between $t_{1/2}$ values for red cell sap samples collected at 1 hr postinjection and plasma samples, which contained no protein bound arsenic. However, $t_{1/2}$ values for 3 and 6 hr red cell sap samples do show a dialyzable, protein bound arsenic component. Exhaustive dialysis and the ethanol precipitation method both revealed arsenic tightly bound to protein in red cell samples.

TABLE 3
THE ARSENIC METABOLITES IN MATERNAL RED CELL SAP AT DIFFERENT TIMES FOLLOWING A SINGLE INTRAPERITONEAL INJECTION OF ARSENATE 8 AM ON THE EIGHTH DAY OF GESTATION

Time postinjection (hr)	No. of samples	Total arsenic concentration (μmoles/kg)	Percentage total arsenic concentration				
			As(V)	As(III)	As_i	DMA	AsP
0.25	2	34.3 ± 1.5	92 ± 3	3	—	2	6 ± 2
0.50	1	100	90	5	—	2	3
0.53	1	99.8	78	8	—	1	13
0.63	1	72.1	79	14	—	2	5
1.0	2	29.6 ± 2.0	—	—	82 ± 4	6 ± 2	13 ± 3
2.0	4	24.2 ± 2.6	77 ± 3	8 ± 1	—	4 ± 1	12 ± 1
3.0	1	11.9 ± 3.7	—	—	83	4	14
4.0	1	11.5 ± 1.7	—	—	83	4	13
5.0	3	6.45 ± 0.75	46 ± 2	12	—	7	35 ± 2
6.0	1	7.56 ± 1.19	—	—	62	20	18

Note. The dose level was 64.2 μmole/kg dam. Assays for low-molecular-weight arsenic and arsenic bound to protein at 1, 3, 4, and 6 hr used the dialysis method, otherwise the ethanol precipitation method was employed (see Materials and Methods). Values represent mean ± standard errors.

The contribution of AsP to the total concentration of arsenic increases with time (Table 2). However, the dialysis method yielded consistently lower values for AsP. Possibly some protein bound arsenic was released on prolonged dialysis by the catalytic action of traces of enzymes or by hemoglobin itself. The origin and significance of red cell AsP are discussed below.

3. Arsenic Metabolites in Maternal Plasma and Red Cells

Arsenate was the dominant plasma arsenic species prior to 4 hr postinjection with only traces of arsenite and DMA present (see Table 2). The decline in the contribution of arsenate beyond 3 hr postinjection was compensated by a rise in the arsenite contribution, indicating active reduction of arsenate. However, DMA was the more dominant metabolized form of arsenate at all sample times. Obviously dimethylation of arsenic is quickly initiated in the hamster following an acute intraperitoneal injection of arsenate.

Rapid reduction and methylation of arsenate by acutely treated animals have been previously reported. Vahter and Envall (1983) reported that the urine of mice and rabbits contained arsenite and DMA 2–3 hr after an intravenous injection of 128 mole arsenate/kg body wt. Vahter, in a review paper (1983), cites her own work and that of others as evidence that methylation in inorganic arsenic is dose dependent. For example, 1 hr after injecting mice intravenously with 128 mole arsenate/kg body wt, plasma arsenic was 32% arsenate, 7% arsenite, and 60% DMA. When the arsenate dose was increased to 12.8 μmole/kg body wt plasma arsenic was 77% arsenate, 7% arsenite, and 15% DMA 1 hr postinjection. Our findings support Vahter's point. Hamster plasma arsenic was 93% arsenate, 2% arsenite, and 5 DMA 1 hr after an intraperitoneal injection of 64.2 μmole arsenate/kg body wt.

Red cell sap arsenic differs significantly from plasma arsenic (see Table 3). The

arsenate contribution is less than that in plasma, arsenite is present prior to 1 hr postinjection, and DMA remains the smallest contributor to low-molecular-weight arsenic throughout the first 6 hr postinjection. The higher concentrations of arsenite and lower concentrations of arsenate in red cell sap samples most likely result from more rapid uptake of arsenite by red cells, assuming Vahter's (1981) *in vitro* data apply. The early appearance of arsenite in red cells (15 min postinjection) is particularly significant in light of the equally early appearance of arsenic tightly bound to protein. The reactivity of arsenite and its participation in nucleophilic displacement reactions (Squibb and Fowler, 1983) makes it likely that AsP results from the interaction of arsenite and sulfhydryl groups of hemoglobin. The stability of the AsP species is indicated by its increasing contribution to total arsenic red cell sap and by relatively little decrease in its absolute contribution over the 1–6 hr after injection (see Fig. 2).

The specific form of arsenic that provokes the teratogenic response is not known. The low teratogenic potential of DMA when administered acutely to mice (Hood, 1972; Willhite, 1981) or chronically to hamsters (V. H. Ferm, unpublished data) makes it an unlikely candidate. Arsenate itself could be the teratogenic species. But, the chemistry of arsenate appears to restrict it to the role of competitive inhibitor in phosphate-dependent enzyme systems and as McCabe and coworkers (1983) point out, the high phosphate concentration in most mammalian cells probably precluded enzyme inhibition by the relatively low arsenate concentrations found in tissues of animals which have received toxic (or teratogenic) doses of arsenate. Arsenite, on the other hand, is known to participate in many nucleophilic displacement reactions with compounds found in biological systems (Squibb and Fowler, 1983). In particular, arsenite could interact with specific enzymes, other proteins, or nucleic acids (Kay, 1965) in differentiating cells to produce fetal abnormalities. The findings of Hood and Bishop (1972) that acutely administered arsenite at 64 μmole/kg body wt in mice is as teratogenic as 143 μmole of arsenate does suggest that arsenite is more nearly related to the actual teratogenic species of arsenic.

Our findings in this investigation can be compared with those obtained in a study of the concentration and chemical status of arsenic in maternal plasma and red cells of hamster dams on the eighth day of gestation resulting from the chronic administration of arsenate (Hanlon and Ferm, 1986). In that study we used the osmotic minipump to provide a constant rate exposure to a teratogenic dose of arsenate. We found that a steady state existed for the concentration and chemical status of arsenic in plasma and red cells during the period of organogenesis (Day 8 of gestation) if the pumps were implanted no later than Day 6 of gestation. It is tempting to ascertain the time interval post-acute injection during which the total concentration and chemical status of arsenic in maternal blood are close to those obtained under conditions known to lead to fetal abnormalities, i.e., those found in the chronic study. In fact, these criteria are met 3 and 4 hr post-acute injection. Our conjecture is that the teratogenic event is initiated no later than 4 hr postadministration. This is consistent with the likelihood that neural tube defects occur early in the period of organ development in hamsters (Ferm and Hanlon, 1983).

4. *Concluding Remarks*

The potential for environmental agents to cause biological damage to mammals is often deduced by assaying the total concentration of the agent in the blood of exposed individuals. The validity of this maneuver is limited by the extent to which the total concentration of arsenic reflects that of the chemical form (or forms) of the agent that is directly responsible for producing a biological insult. In turn, the blood concentration and chemical status of a given agent at any time following the initiation of exposure must be dependent on the intensity, mode, and duration of exposure as well as the intrinsic genetically determined metabolic possibilities provided by the exposed individual. Therefore, without knowledge of the history of the exposure, judgments regarding toxic or teratogenic outcomes founded on the total concentration of a suspected environmental agent may well be baseless.

Our investigations concerning the metabolic fate of teratogenic doses of arsenate administered under acute and chronic conditions provide a firmer base for predicting the teratogenic potential of arsenate in mammals and could be useful for predicting the likelihood of fetal abnormalities in humans following exposure to arsenate in the environment.

ACKNOWLEDGMENTS

We thank Ms. Tracy Keller and Ms. Susan Ferm for their technical assistance. This project has been financed in part with Federal funds from the Environmental Protection Agency under Grant R811078. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency nor does mention of trade names or commercial products constitute endorsement of recommendation for use. Additional support was provided by USPHS Grant OH-01434.

REFERENCES

Bertolero, F., Marafante, E., Rade, J., Pietra, R., and Sabbioni, E. (1981). Biotransformation and intracellular binding of arsenic in tissues of rabbits after intraperitoneal administration of ^{74}As-labeled arsenite. *Toxicology* **20,** 35–44.

Ferm, V. H. (1965). The rapid detection of teratogenic activity. *Lab. Invest.* **14,** 1500–1505.

Ferm, V. H., and Carpenter, S. J. (1968). Malformations induced by sodium arsenate. *J. Reprod. Fertil.* **17,** 199–201.

Ferm, V. H., and Hanlon, D. P. (1983). Metal-induced congenital malformations. *In* "Reproductive and Developmental Toxicity of Metals" (T. W. Clarkson, G. F. Nordberg, and P. Sager, Eds.), pp. 383–398. Plenum, New York.

Ferm, V. H., and Hanlon, D. P. (1985). Constant rate exposure of pregnant hamsters to arsenate during early gestation. *Environ. Res.* **37,** 425–432.

Hanlon, D. P., and Ferm, V. H. (1977). Placental permeability of arsenate ion during early embryogenesis in the hamster. *Experientia* **33,** 1221–1222.

Hanlon, D. P., and Ferm, V. H. (1986). Concentration and chemical status of arsenic in the blood of pregnant hamsters during critical embryogenesis. 1. Subchronic exposure to arsenate utilizing constant rate administration. *Environ. Res.* **40,** 372–379.

Hood, R. D. (1972). Effects of sodium arsenite on fetal development. *Bull. Environ. Contam. Toxicol.* **7,** 216–222.

Hood, R. D., and Bishop, S. L. (1972). Teratogenic effects of sodium arsenate in mice. *Arch. Environ. Health* **24,** 62–65.

Kay, E. R. M. (1965). Incorporation of radio-arsenate into proteins and nucleic acids of Erlich lettre ascites carcinoma *in vitro*. *Nature* (*London*) **206,** 371–373.

Lindgren, A., Danielsson, B., Dencker, L., and Vahter, M. (1984). Embryotoxicity of arsenite and

arsenate: Distribution in pregnant mice and monkeys and effects on embryonic cells *in vitro*. *Acta Pharmacol. Toxicol.* **54,** 311–320.

McCabe, M., Maguire, D., and Nowak, M. (1983). The effects of arsenic compounds on human and bovine lymphocyte mitogenesis *in vitro*. *Environ. Res.* **31,** 323–331.

Morrissey, R. E., and Mottet, N. K. (1983). Arsenic-induced exencephaly in the mouse and associated lesions occurring during neurulation. *Teratology* **29,** 339–411.

Pomeroy, 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.

Squibb, K. S., and Fowler, B. A. (1983). The toxicity of arsenic and its compounds (Chap. 7). *In* "Topics in Environmental Health," Vol. 6, "Biological and Environmental Effects of Arsenic" (B. A. Fowler, Ed.). Elsevier, New York.

Tam, K. H., Charboneau, S. M., Bryce, F., and Lacroix, G. (1978). Separation of arsenic metabolites in dog plasma and urine following intravenous injection of ^{74}As. *Anal. Biochem.* **86,** 505–511.

Vahter, M. (1981). Biotransformation of trivalent and pentavalent inorganic arsenic in mice and rats. *Environ. Res.* **25,** 286–293.

Vahter, M. (1983). Metabolism of arsenic (Chap. 5). *In* "Topics in Environmental health," Vol. 6, "Biological and Environmental Effects of Arsenic" (B. A. Fowler, Ed.). Elsevier, New York.

Vahter, M., and Envall, J. (1983). *In vivo* reduction of arsenate in mice and rabbits. *Environ. Res.* **32,** 14–34.

Vahter, M., and Norin, H. (1980). Metabolism of ^{74}As-labeled trivalent and pentavalent inorganic arsenic in mice. *Environ. Res.* **21,** 446–457.

Willhite, C. C. (1981). Arsenic-induced axial skeletal (dysraphic) disorders. *Exp. Mol. Pathol.* **34,** 145–158.