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Prenatal Toxicity of Orally Administered Sodium Arsenite in Mice

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Biological consequences of the increasing introduction of arsenic into the environment have not been fully investigated. Exposure to arsenic compounds may occur from sources such as herbicides (SCHWEIZER 1962), insecticides (BISHOP and CHISOLM 1962), rodenticides, paint pigments (ANONYMOUS 1974), and wood preservatives (NELSON 1977). Environmental contamination by arsenic may also occur from the wastes derived from the production of copper, lead, and zinc, and as a by-product of the use of fossil fuels (NELSON 1977).

It has been shown that arsenic is teratogenic in chicks (PETERKOVA and PUZANOVA 1976), golden hamsters (FERM and CARPENTER 1968), mice (HOOD and BISHOP 1972), and rats (BEAUDOIN 1974). There has also been at least one report of neonatal death resulting from ingestion of arsenic by a pregnant woman (LUGO et al. 1969). In most of these studies, the form of arsenic studied was arsenate (As^{+5}). Just two such studies (HOOD 1972; PETERKOVA and PUTZANOVA 1976) were concerned with arsenite (As^{+3}), and there has been only one preliminary report (MATSUMOTO et al. 1973) dealing with orally administered arsenite.

HOOD et al. (1972) reported that sodium arsenite administered intraperitoneally to pregnant mice at dose levels that were toxic to the dams (10 or 12 mg/kg) was embryolethal; some maternal deaths were also reported. Treatment with arsenite on days 8, 9, or 10 induced gross and skeletal malformations similar to those reported previously for comparably toxic levels of arsenate given to mice (HOOD and BISHOP 1972).

MATSUMOTO et al. (1973) treated an unspecified number of pregnant ICR mice by gavage on days 10, 11 and 12 with 10, 20 or 40 mg/kg sodium arsenite. They reported increased prenatal mortality and decreased fetal weights in the high dose groups, as well as a low incidence of malformations at 10 and 40 mg/kg (6 and 4%, respectively). The reported incidence of malformations thus did not appear to be dose-dependent.

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There is a lack of information on the prenatal effects of maternal oral exposure to trivalent arsenic. Because the oral route is the most common route for environmental arsenic exposure, the present study investigated the effects of orally administered arsenite on fetal development, using the mouse as an animal model.

MATERIALS AND METHODS

All trials were done with CD-1 mice (Charles River Breeding Laboratories) fed Wayne Lab Blox and fresh tap water ad libitum. Sexually mature (26-30 g) mice were mated, and the day on which a vaginal plug was noted was designated gestation day 1 (all literature data discussed have been adjusted to conform to this terminology). Arsenic treatment consisted of a single dose of 20, 40, or 45 mg/kg sodium arsenite (Fisher Scientific) in distilled H_2O given by gavage. Arsenic content was verified by hydride generation atomic absorption spectrophotometry. The dose volume was 0.01 ml/g of body weight. Treatments were given on one of days 8-15 of pregnancy. Controls were given equivalent volumes of H_2O or were untreated.

On gestation day 18, the dams were weighed and sacrificed by cervical dislocation. Litters were evaluated for prenatal mortality, and all live fetuses were examined for gross malformations and weighed. Each arsenic-treated and solvent-control litter was divided randomly so that one third of the fetuses in the litter were subjected to visceral examination by the method of STAPLES (1974), and 2 grossly normal fetuses were fixed in chilled 10% buffered formalin for examination by light microscopy. The formalin-fixed fetuses were processed by a modification of the method of JONES et al. (1971). A transverse section through the head was removed so that the eyes and brain could be embedded in the same block used for the remainder of the carcass. A whole body sagittal section through the carcass of each fetus usually included all parenchymatous organs (including the gonads) except the spleen. All sections were stained with hematoxylin and eosin. The remaining fetuses were cleared and stained by CRARY'S (1962) method for visualization of skeletal malformations. Maternal liver/body weight ratios were determined, along with average maternal food consumption.

Statistical analyses on weight data were done by ANOVA followed by SNK multiple range tests (SOKAL and ROHLF 1969). Percentage data were analyzed by the nonparametric method of WILCOXON and WILCOX (1964).

RESULTS AND DISCUSSION

The lowest dose of sodium arsenite (20 mg/kg) produced no discernible teratogenic or maternal toxic effects in 8 to 15 pregnant mice exposed per treatment day, and the data are not presented in detail. With the 40 and 45 mg/kg arsenite doses, maternal mortalities were 19 and 36%, respectively, and prenatal effects were observed. These data are presented in Table 1. At 40 mg/kg, a low incidence of gross malformations, consisting of exencephaly (with related facial anomalies) and open eyes, was noted only in fetuses from dams treated on days 8 or 9. Similar malformations were observed in fetuses from the dams treated with 45 mg/kg, if exposure to arsenic was on the 8th, 9th, or 10th day of gestation.

The low incidence of malformations observed contrasts with the results of HOOD (1972), with sodium arsenite given to mice ip. Following treatment at 12 mg/kg in the earlier study, there were 0, 27 and 36% grossly abnormal fetuses when exposure was on days 8, 9, or 10, respectively. The types of gross malformations noted also differed in the two studies. Intraperitoneal treatment resulted in a greater variety of anomalies, such as umbilical hernias and micrognathia. In a similar study by HOOD and BISHOP (1972) on sodium arsenate given ip, a similar variety of anomalies was seen following treatment on days 8, 9, or 10, at doses of 45 mg/kg.

In a comparison of average fetal body weights, no significant differences were found between the control fetuses and those from dams given 40 mg/kg arsenite. At the 45 mg/kg dose, however, there was a significant decrease in fetal body weights when exposure occurred on days 9, 10, 14, or 15. These data can be compared to previous results with arsenite administered ip. HOOD (1972) found that fetuses from dams treated on one of days 7, 8, 10, or 11 (but not days 9 or 12) at 10 mg/kg had significantly lower body weights than did fetuses from control dams; at 12 mg/kg there was an even greater effect.

The results of the current study are in agreement with those previously reported by HOOD et al. (1978), who observed that sodium arsenate orally administered to CD-1 mice had a smaller effect on fetal body weight than did the same compound given ip. In both studies, the highest dose levels used were similary toxic to the dams, but maternal oral treatment tended to result in less effect on the fetus than did ip injection.

By far the greatest indication of arsenite's effect on the fetus was prenatal mortality. At the 40 mg/kg dose, fetal mortality was increased significantly for litters from dams treated by gavage on days 10 or 12. At the high dose (45 mg/kg) increased fetal mortality was observed in litters from dams treated on days 10, 12, 13, 14, or 15.

TABLE 1

EFFECTS ON MOUSE DEVELOPMENT OF MATERNAL ORAL EXPOSURE TO SODIUM ARSENITE ON ONE OF GESTATION DAYS 8-11.

Treatment[1]		Dose	Litters Examined	Maternal Deaths	Total Implantations	Fetal Body Wt. (G)	Incidence of Gross Malformations		Dead or Resorbed
Day	Type	(mg/kg)	(#)	(#)	($\bar{X}$)	$\bar{X}$ ± (SD)	No.	(%)	(%)
8	As^{+3}	40	20	2	12.9	1.07 (0.01)	3/244	1.2	5.4
		45	5	1	12.6	0.99 (0.02)	1/60	1.7	4.8
	H_2O		16	1	11.9	1.08 (0.01)	0/178	0.0	6.8
9	As^{+3}	40	14	3	11.9	1.02 (0.02)	6/157	3.8	6.0
		45	4	3	14.2	0.86 (0.01)*	5/55	9.1	3.5
	H_2O		14	0	12.4	1.02 (0.01)	0/165	0.0	5.2
10	As^{+3}	40	15	1	12.3	1.05 (0.02)	0/121	0.0	34.6**
		45	8	1	12.4	0.91 (0.01)*	2/77	2.6	22.2*
	H_2O		18	0	11.1	1.02 (0.01)	0/193	0.0	3.5
11	As^{+3}	40	18	6	12.4	1.04 (0.01)	0/196	0.0	12.1
		45	6	5	13.3	0.97 (0.01)	0/71	0.0	11.2
	H_2O		17	0	12.3	1.01 (0.01)	0/199	0.0	4.8

1 Given by gastric intubation on the indicated gestation day.

*,** Significantly different from solvent (H_2O) control ($P<0.05$ or 0.01, respectively).

TABLE 1 (Continued)

EFFECTS ON MOUSE DEVELOPMENT OF MATERNAL ORAL EXPOSURE TO SODIUM ARSENITE ON ONE OF GESTATION DAYS 12-15.

Treatment[1]		Dose	Litters Examined	Maternal Deaths	Total Implantations	Fetal Body Wt. (G)	Incidence of Gross Malformations		Dead or Resorbed
Day	Type	(mg/kg)	(#)	(#)	($\bar{X}$)	$\bar{X}$ ± (SD)	No.	(%)	(%)
12	As^{+3}	40	20	3	12.4	1.01 (0.01)	0/204	0.0	17.7*
		45	12	5	12.0	0.98 (0.01)	0/104	0.0	27.8*
	H_2O		16	0	12.7	0.95 (0.01)	0/196	0.0	3.4
13	As^{+3}	40	16	4	12.2	1.05 (0.01)	0/170	0.0	12.8
		45	7	1	12.0	0.94 (0.02)	0/39	0.0	53.6**
	H_2O		17	0	11.2	1.07 (0.01)	0/184	0.0	3.2
14	As^{+3}	40	16	5	12.5	0.99 (0.01)	0/180	0.0	10.0
		45	5	6	11.2	0.80 (0.02)*	0/36	0.0	35.7*
	H_2O		18	0	12.3	1.05 (0.01)	1/211	0.5	4.5
15	As^{+3}	40	16	7	12.4	0.97 (0.01)	0/174	0.0	12.1
		45	6	7	12.3	0.91 (0.01)*	0/49	0.0	33.8*
	H_2O		17	0	11.6	1.06 (0.01)	0/189	0.0	4.1
Untreated			21	0	11.8	1.03 (0.01)	0/236	0.0	4.8

1 Given by gastric intubation on the indicated gestation day.

*,**Significantly different from solvent (H_2O) control ($P<0.05$ or 0.01, respectively).

In the study by HOOD (1972), sodium arsenite was also embryolethal when given to pregnant mice ip. The greatest effect was seen at the high (12 mg/kg) dose level. The observed prenatal mortality was generally greater in the previous study than in the current one. HOOD et al. (1978) reported more prenatal deaths in litters from dams given sodium arsenate by ip injection (40 mg/kg) than in those from females treated by gavage (120 mg/kg). This occurred concurrent with an almost equivalent rate of maternal mortality for the two treatments and again agrees in trend with the current results.

Visceral examinations and light microscopy done on fetuses from mice in the current study (with trivalent arsenic) are consistent with the findings of HOOD et al. (1978), who used the pentavalent form. Both studies suggest that arsenic does not cause visceral malformation in the mouse. This is in contrast to the reports of renal agenesis and other visceral abnormalities noted in the rat (BEAUDOIN 1974) and the hamster (FERM et al. 1971) following arsenate treatment. Also, although most organs were present in the whole body sections from fetuses examined by light microscopy, no treatment-related pathoanatomic alterations were observed regardless of the day of gestation on which exposure occurred.

Skeletal analysis revealed no treatment effect. A pair of fused ribs in a fetus from a dam treated on day 10 at the high dose was the only malformation noted. These findings differ from those of the study by HOOD (1972), in which a significant incidence of defective ribs was noted following arsenite administration by ip injection on days 9 or 10.

Food consumption was decreased immediately following arsenite treatment and returned to normal levels on subsequent days. There were no effects on maternal liver/body weight ratios.

Since arsenite is the more toxic form, arsenite should be fetotoxic at lower dose levels than would be the case with arsenate. This assumption is supported by a comparison of the effective doses reported herein (40 or 45 mg/kg) with those reported previously by HOOD et al. (1978) with oral arsenate (120 mg/kg).

Although the exact mechanism of teratogenesis is unknown, it is known that arsenite affects several major cellular metabolic pathways. For example, arsenite is thought to be a strong inhibitor of the β-oxidation of fatty acids because of its inhibition of thiolases (REIN et al. 1979). Arsenite is known to be a sulfhydryl poison, reacting with the sulfhydryl groups of a variety of proteins (COMMITTEE ON MEDICAL AND BIOLOGICAL EFFECTS 1977). It also inhibits the oxidation of pyruvate for the formation of acetyl CoA (an obligatory step for entry of carbohydrates into the Krebs Cycle) by binding to lipoic acid

(HARVEY 1975). Thus, arsenite may inhibit fetal energy metabolism and may interfere with other metabolic processes.

The differences noted between the adverse effects of po and ip treatments may involve several factors. Arsenic compounds must be absorbed into the blood stream significantly more slowly when given orally than when given ip, in order to account for the relatively greater toxicity of ip arsenic. This is particularly likely in view of the report of VAHTER and NORIN (1980), which indicates that in mice virtually all of an orally administered dose of inorganic arsenite or arsenate is ultimately absorbed from the gut. If oral treatment results in a significantly reduced rate at which arsenic enters the bloodstream and reaches the conceptus, the peak level acting on the developing system should be lower and the effect thus could be diminished. Preliminary findings indicate that both arsenite and arsenate, whether given po or ip, do reach the day 18 mouse fetus in readily quantifiable amounts (HOOD et al., unpublished data). This finding would be expected in view of the ability of small molecules to cross the placenta. Data for younger fetuses, however, is not yet available.

It is also known that the mouse, as well as man and certain other mammals, methylates arsenic (CRECELIUS 1977; ODANAKA et al. 1980). This process should significantly reduce arsenic's toxicity (CHRISTENSEN and LUGINBYHL 1974). Thus, maternal methylation could also aid in reducing the adverse effect of arsenic on the conceptus. If orally administered arsenic is methylated to a greater extent than is injected arsenic this could result in differential effects on the conceptuses of dams given arsenic by different modes of administration. This seems unlikely, however, as the data of ODANAKA et al. (1980) indicate similar levels of methylation in mice following po and iv arsenic administration.

These results suggest that prenatal exposure to maternally ingested arsenite is less likely to be harmful than would be expected from previous studies of injected arsenite in mice (HOOD 1972). In preliminary trials with arsenite gavaged hamsters, however, HARRISON and HOOD (unpublished data) found high incidences of prenatal death at dose levels of only 25mg/kg. Studies with two species thus indicate that the adverse effects of oral arsenite on the mother and fetus can be achieved at significantly lower doses than is the case with oral sodium arsenate (HOOD et al., 1978).

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