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Constant Rate Exposure of Pregnant Hamsters to Arsenate during Early Gestation

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We have examined the teratogenic and embryotoxic effects of constant-rate exposure of pregnant hamsters to arsenate by means of subcutaneous implants of osmotic minipumps. Different total exposure regimens were established by varying the duration of minipump implants and by varying the concentration of arsenate in the minipumps. Dams were killed on Day 13 of pregnancy, 5 days after the critical stage of organogenesis. Numbers of resorptions, dead fetuses, and living fetuses were obtained. Fetal weights, crown–rump lengths, and the incidence of malformations were recorded. Control animals were treated identically with minipumps containing demineralized water. The percentage of malformations per litter, a direct measure of teratogenesis, was dependent only upon the concentration of arsenate in the minipumps. The minimum teratogenic response was achieved with a dose of 70 μmol/kg dam/24 hr during the critical stages of organogenesis. The embryotoxic (fetotoxic) indicators, fetal weight and crown–rump length, decreased with increases in exposure time and with increased concentrations of arsenate. The resorption rate also depended directly upon duration of exposure and concentration of arsenate in the minipump.

INTRODUCTION

Teratogens can affect embryonic development by acute or chronic exposure. While acute accidental exposure may often be a well documented event, the usual exposure to a potential environmental teratogen is more insidious and chronic in nature.

There have been relatively few attempts to measure the effects of chronic exposure of teratogenic agents in animal model systems. Of these, the delivery of such potential agents has usually been through food or drinking water exposure (Schroeder and Mitchener, 1971). Recently, the constant-rate administration of sodium cyanide utilizing the osmotic minipumps has demonstrated the teratogenic capabilities of that compound (Doherty *et al.*, 1982). Nan *et al.* (1981) have studied valproic acid toxicity in pregnant mice by the same technique.

Arsenic has been identified as a significant teratogen in animal model systems when administered in acute bolus doses (Ferm *et al.*, 1971; Willhite and Ferm, 1983). The present experiments have been designed to investigate the subchronic exposure of pregnant hamsters to the constant-rate administration of arsenic by implanting subcutaneous osmotic minipumps (Alzet) containing sodium arsenate during four stages of early gestation. These pumps deliver the arsenic at a constant rate, and both the concentration and the length of exposure to the teratogen can be easily controlled. Quantitative data on the amount of exposure to potential environmental teratogens together with the minimal circulating blood levels of

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such agents required to produce an effect on the developing embryo would be extremely useful. This paper details the effect of arsenic, delivered by constant-rate exposure, on a number of reproductive parameters during gestation.

MATERIALS AND METHODS

Timed pregnant hamsters (LGV strain) were obtained from the Charles River Laboratories (Wilmington, Mass.). The protocol for the breeding, timing, and the critical stages of embryogenesis in this species have been described (Ferm, 1967).

Preparation of pumps. Osmotic minipumps (Alzet; Model 2001) were used in these experiments. Each pump was weighed and then filled with a solution containing 150, 175, 200, 225, or 250 mg/ml of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) made up in demineralized water. Each pump was reweighed after filling to assure that it had been properly filled. The pumps were placed into a normal saline solution for a period of 4 hr to activate the osmotic process (upon activation the pumps release 1 μl/hr) and then implanted into pregnant hamsters. For controls, some pumps were filled with demineralized water.

Insertion of pumps. On Days 4–7 of gestation (see Fig. 1) maternal animals were anesthetized by i.p. injection of 6.5 mg of sodium pentobarbital per 100 g of weight. The back of the anesthetized animal was shaved with clippers and cleansed with 70% alcohol; a 1.5-cm incision was made through the skin in the midline. The pump was inserted into a subcutaneous location with the pumping end diverted away from the site of incision. The skin was closed with skin clips and the animal returned to its cage. Control pumps containing demineralized water were inserted on Days 4 and 6 of gestation.

Data collection. The maternal animals were weighed daily. On the 13th day of gestation, 5 days after the critical stages of embryogenesis in this species (Ferm, 1967), the maternal animals were killed and weighed, and their uteri were examined. Resorption sites, evidence of embryonic or fetal death, were recorded. The living fetuses were examined for external malformations and then weighed and measured for crown–rump length. Fetuses from mothers who served as con-

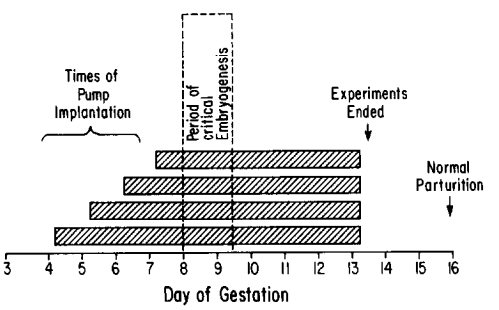

FIG. 1. Experimental design showing times of exposure of pregnant hamsters to osmotic minipumps containing sodium arsenate. The critical period of embryogenesis in this species is shown in relation to these exposures.

trols were collected also on Day 13 and examined in the same way as the experimental groups.

RESULTS

We grouped the animals into five dose ranges because the weights of the maternal animals varied, while the concentration of arsenate within the pumps was kept at the five concentrations. For each of these dose groups the following calculations were made: (1) the number of millimoles of arsenate received during the 24-hr period comprising the 8th day of gestation; (2) the total amount of arsenate (millimoles) received from the time of minipump implantation through Day 9.5 (the end of the critical embryogenetic period); and (3) the total amount of arsenate (millimoles) received from the time of insertion of the pump until the animal was sacrificed on Day 13. The dose ranges for each of these periods of exposure for each of the five groups are given in Table 1.

Because two variables, time and dose, have been used in these experiments we chose to discuss the data (Table 2) under the following headings: constant dose–variable time (CD-VT), and variable dose (concentration of arsenate in

TABLE 1
DOSE OF SODIUM ARSENATE

Treatment group	Implant day	Day 8 (24 hrs) (critical stage of embryogenesis)	Implantation to Day 9½ (end of critical stage of embryogenesis)	Implantation to Day 13 (end of experiment)
I	4	0.0730–0.0789	0.3474–0.3871	0.6192–0.6886
	5	0.0709–0.0817	0.2747–0.3197	0.5474–0.6329
	6	0.0682–0.0808	0.1979–0.2361	0.4594–0.5447
	7	0.0710–0.0764	0.1375–0.1537	0.4121–0.4470
II	4	0.0813–0.0903	0.3994–0.4440	0.7112–0.7903
	5	0.0825–0.0913	0.3317–0.3661	0.6482–0.7163
	6	0.0844–0.0928	0.2438–0.2681	0.5675–0.6239
	7	0.0836–0.0903	0.1672–0.1724	0.4878–0.5187
III	4	0.0940–0.0993	0.4642–0.4907	0.8247–0.8714
	5	0.0948–0.1081	0.3714–0.4180	0.7350–0.8328
	6	0.1033–0.1039	0.2933–0.3158	0.6780–0.7142
	7	0.0931–0.1092	0.1773–0.2204	0.5344–0.6392
IV	4	0.1006–0.1088	0.4963–0.5357	0.8820–0.9532
	5	0.1092–0.1154	0.4298–0.4495	0.8486–0.8891
	6	0.1086–0.1194	0.3190–0.3548	0.7354–0.8126
	7	0.1110–0.1175	0.2198–0.2334	0.6495–0.6793
V	4	0.1169–0.1447	0.5721–0.7102	1.0239–1.2650
	5	0.1179–0.1555	0.4563–0.6005	0.9085–1.1967
	6	0.1264–0.1373	0.3605–0.3914	0.8453–0.9178
	7	0.1239–0.1317	0.2445–0.2662	0.7249–0.7713

Note. Amount of arsenate delivered is calculated as millimoles/kilogram maternal weight for the times indicated.

TABLE 2
EFFECT OF CONSTANT-RATE ADMINISTRATION OF SODIUM ARSENATE ON FETAL DEVELOPMENT

Group	Day	No. of mothers	No. of gestation sacs	Resorptions (no. (%))	Living fetuses (no. (%))		Crown–rump length (mm; mean ± SEM)	Fetal wt. day 13 (g; mean ± SEM)
					Normal	Malformed		
Control	4	11	162	4 (2.46)	157 (99.3)	1 (0.6)	16.3 ± 0.1	0.535 ± 0.006
	6	10	139	2 (1.43)	136 (99.2)	1 (0.8)	16.1 ± 0.1	0.489 ± 0.007
I	4	5	75	8 (10.7)	63 (94.0)	4 (6.0)	14.6 ± 0.1	0.372 ± 0.009
	5	6	103	4 (3.9)	82 (82.8)	17 (17.2)	14.4 ± 0.1	0.379 ± 0.007
	6	7	106	9 (8.5)	89 (91.7)	8 (8.3)	14.8 ± 0.1	0.425 ± 0.007
	7	6	92	3 (3.3)	82 (92.1)	7 (7.9)	15.4 ± 0.1	0.472 ± 0.008
II	4	6	99	23 (23.2)	55 (72.4)	21 (27.6)	14.0 ± 0.1	0.341 ± 0.008
	5	6	95	13 (24.4)	62 (75.6)	20 (24.4)	14.0 ± 0.1	0.348 ± 0.007
	6	7	102	10 (9.8)	72 (78.3)	22 (21.7)	14.8 ± 0.1	0.413 ± 0.008
	7	6	86	7 (8.1)	60 (75.9)	19 (24.1)	15.1 ± 0.1	0.433 ± 0.009
III	4	6	76	47 (61.8)	16 (55.2)	13 (44.8)	13.8 ± 0.1	0.305 ± 0.009
	5	7	103	48 (46.6)	37 (67.3)	18 (32.7)	14.4 ± 0.2	0.360 ± 0.012
	6	7	109	54 (49.5)	27 (49.0)	28 (50.9)	14.1 ± 0.1	0.326 ± 0.006
	7	7	110	25 (22.7)	60 (70.6)	25 (29.4)	14.9 ± 0.1	0.437 ± 0.007
IV	4	5	77	46 (59.7)	12 (38.7)	19 (61.3)	13.7 ± 0.2	0.315 ± 0.009
	5	8	125	77 (61.6)	24 (50.0)	24 (50.0)	14.1 ± 0.1	0.325 ± 0.009
	6	6	90	51 (56.7)	27 (69.2)	12 (30.8)	14.5 ± 0.2	0.387 ± 0.008
	7	6	80	16 (20.0)	36 (56.2)	28 (43.7)	14.4 ± 0.1	0.390 ± 0.011
V	4	8	110	95 (86.4)	5 (33.3)	10 (66.7)	13.1 ± 0.2	0.263 ± 0.009
	5	6	93	80 (86.0)	3 (23.0)	10 (76.9)	14.0 ± 0.1	0.305 ± 0.010
	6	5	68	31 (45.6)	28 (75.7)	9 (24.3)	14.6 ± 0.1	0.380 ± 0.008
	7	5	73	31 (42.5)	13 (30.9)	29 (69.0)	15.0 ± 0.2	0.415 ± 0.009

minipump)–constant time (VD-CT). Under these conditions the hamster model shows the following responses to arsenate in our test system.

1. Effects of Arsenic on Fetal Weight

CD-VT mode. When the dose level of arsenic administered remains relatively constant within a treatment group (see Table 1), the exposures of greater duration result in lower fetal weights at the end of the experimental period (Day 13). These changes are significant in all five of the treatment groups.

VD-CT mode. Increase in dose levels administered during the same day of gestation correlate with decreases in fetal weight, although the correlation is not as strong as with the CD-VT data.

2. Effects of Arsenic on Fetal Crown–Rump Length

CD-VT mode. There is no markedly obvious trend in this mode of treatment although there is the suggestion that the longer the exposure of the fetus to arsenic, the smaller the crown–rump length will be.

VD-CT mode. There is relatively little discernable effect on crown–rump length in this treatment mode except for Day 4 implants which show a progressive decrease in length with increasing dose.

3. Effect of Arsenic on Fetal Resorptions (Embryonic or Early Fetal Death)

The data on fetal resorption and malformations are summarized in Table 2 and depicted in Fig. 2.

CD-VT mode. Groups I–IV show an increasing resorption rate with duration of exposure. Within these groups the pumps implanted on Day 7 showed the least effect on fetal resorption.

VD-CT mode. There was a strong correlation between dose and resorption rate among the five treatment groups.

4. Effect of Arsenic on Congenital Malformations

The types of malformations seen in these experiments were similar in organ specificity to those previously described for arsenic administered intravenously or intraperitoneally (Ferm, 1977). These malformations are severe neural tube defects including exencephaly and encephaloceles. In addition, a few rib malformations were noted.

CD-VT mode. Within a treatment group there is no obvious difference in the malformation rates when compared to the duration of pump implantation.

VD-CT mode. There is a high degree of correlation between increasing dose and percentage of malformations of surviving fetuses for all time exposure groups. Pumps containing 100 mg/ml implanted on Day 6 of gestation had no statistical effect on fetal weight or crown–rump length compared to controls. In this group of 10 mothers with 155 embryos there were 4 fetuses with very minor malformations (for a malformation rate of 2.7%). The resorption rate in this group was

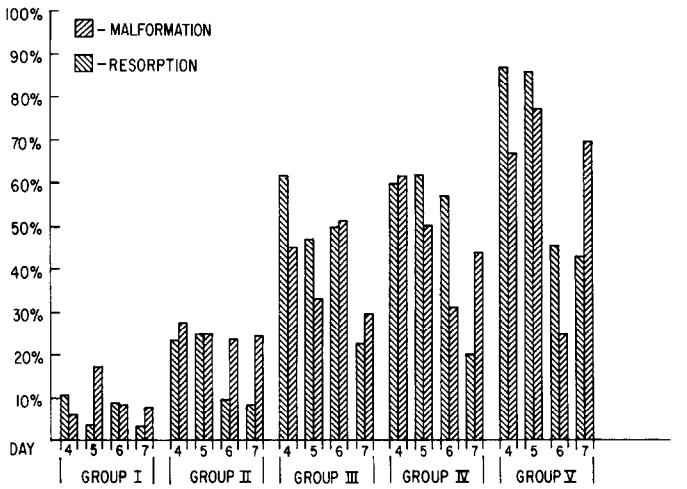

FIG. 2. Rates of congenital malformation and fetal resorption determined at the end of the experimental period on Day 13 of gestation. Each group corresponds to one dose level of sodium arsenate (see text).

not increased over controls. The amount of arsenic which the mother received during the 8th day of gestation ranged from 0.0346 to 0.0538 mmol/kg body wt.

DISCUSSION

There is little doubt about the efficacy of the minipump method in the specific context of arsenate-induced teratogenic lesions in our hamster model. In order to produce a minimal teratogenic response, the concentration of arsenate must reach a certain level at critical sites in the maternal–embryonic system. The dose–dependent increase in percentage malformations shown in Table 2 indicates that this level of arsenate is achieved at 150 mg/ml of arsenate in the minipump, regardless of exposure time, and is exceeded in graded fashion by higher concentrations. From our data it is evident that arsenate delivery above 70 μmol/kg during the 24-hr period of critical embryogenesis (Day 8) in the hamster produces teratogenic results.

The degree of teratogenic response should correlate directly with the concentration of a teratogenic agent at critical stages in the maternal–embryonic system during organogenesis. This critical period in the hamster occurs during the 8th day of gestation (Fig. 1) and the placenta of the hamster is readily permeable to sodium arsenate at this time (Hanlon and Ferm, 1977). The present experiments have shown that there is an increase in the malformation rate among surviving fetuses as the dose of arsenate increases. However, there is no marked trend in the malformation rate with changes in exposure time. In this context the data presented for percentage malformations can be interpreted to mean that increased exposure time does not lead to an increased concentration of arsenate at the critical sites (i.e., arsenate is not stored to any marked degree at these locations)

and that blood levels of arsenate probably reach equilibrium between dose delivered and excretion very rapidly for each dose level.

The detectable malformation rate depends upon the time in gestation that embryos or fetuses are examined. If the embryos are examined too early, certain conclusions about teratogenicity may be misleading. For example, if, in the hamster, the embryos are examined the 9th day of gestation, no conclusions can be made concerning the effect of the teratogen on possible limb bud defects because the limbs are not yet completely formed. On the other hand, if the fetuses are examined for malformations near the end of gestation, those with serious malformations may well have died and begun to undergo resorption. Only careful examination of such embryos immediately after the critical stages of embryogenesis would detect these malformations. Thus fetal resorption is associated with severe developmental malformations and the degree of fetal resorption is commonly used as an index of teratogenic activity. Our data are consistent with the possibility that fetal resorption due to chronic arsenate exposure results from both teratogenic and embryotoxic effects since the degree of fetal resorption is dependent upon the duration of exposure.

Criteria other than teratogenicity which are often used to indicate a possible toxic effect of arsenic on fetal development are fetal mass (weight) and fetal size (crown–rump) measurements. Of these, the crown–rump measurement is probably the most subjective. Both of these criteria taken together indicate an effect of arsenic on growth *in utero*. The site of this effect in the maternal–placental–embryonic unit is not known.

In conclusion, our study demonstrates that the minipump implantation method is a valid approach to the study of teratogenesis provoked by constant–rate dosing during gestation. Its particular value lies in the release of the test agent to the maternal–embryonic unit at a constant rate. This technique should also provide blood levels of environmental agents during chronic exposures to doses which induce teratogenic and/or toxic effects in reproduction.

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