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Teratogen Concentration Changes as the Basis of the Heat Stress Enhancement of Arsenate Teratogenesis in Hamsters

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ABSTRACT Hamster dams dosed continously with arsenate and exposed to short-term hyperthermia produced a greater percentage of malformed offspring than did hamster dams dosed with arsenate alone. Hamsters receiving both treatments possessed elevated arsenic concentrations in the maternal blood and placentas immediately after cessation of the hyperthermic insult. Blood levels of arsenic were the same as those of animals not receiving the heat treatment within several hours post-hyperthermia; however, arsenic concentrations remained elevated in placentas, the duration being dependent on the dose of arsenate. We suggest that the rise in placental arsenic concentrations is the basis of the increase in the production of fetal malformations for hamsters treated continuously with arsenate and heat stressed during critical organogenesis.

Hyperthermia produces developmental abnormalities in a wide variety of mammals if the exposure occurs early in gestation (Edwards, '74). These findings support the speculation that maternal hyperthermia during early gestation may be a causative factor in the production of birth defects in humans (Miller et al., '78; Kleinbracht et al., '79; Fisher and Smith, '81). Immediately related to this issue is the fact that certain teratogens appear to act synergistically with heat exposure in experimental animals. For example, Ferm and Kilham ('77) found that a combination of short-term hyperthermia and acute arsenate dosing early in the period of organogenesis (the morning of day 8 of gestation) produced significantly more fetal abnormalities in hamsters than could be accounted for on the basis of the additive effects of the two treatments.

We have recently reported (Ferm and Hanlon, '85) that malformations produced by continuously dosing hamster dams with arsenate via the osmotic minipump are similar to those seen following acute dosing with arsenate on the morning of day 8 of gestation (Ferm, '77). We have now utilized the osmotic minipump to study the production of teratogenic lesions in the hamster under conditions of continuous exposure to arsenate in combination with short-term maternal heat stress induced during the early phase of the critical period of organogenesis (morning of day 8 of gestation). We have posed two questions, both relevant to the hyperthermia/arsenate combination in the hamster model. First, is the teratogenic effect of arsenate administered continuously via the minipump augmented by a maternal hyperthermic event as it is in the acute exposure regimen? Second, what effect does maternal hyperthermia have on the level of arsenic in maternal blood and in placentas following the heat stress event?

METHODS

Teratogenic studies

Osmotic minipumps (Alzet: model 2001) were charged with 0.321 M or 0.482 M sodium arsenate and implanted in pregnant Syrian hamsters on day 6 of gestation following a method described by Ferm and Hanlon ('85). For both dose regimens one group of animals was placed in a water-jacketed incubator at 39°C for a period of 50 minutes on the morning of day 8 of gestation. A second group received no heat treatment. On day 13 of gestation dams were killed and the fetuses were recovered and examined for developmental malformations by using a protocol devised by Ferm ('67). In addition, embryonic

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resorptions were recorded. The Wilcoxon unpaired rank test was used to determine statistical differences in the proportion of fetuses malformed in each litter for dams receiving different treatments.

Arsenic measurements

Dosing and heat exposures were performed as described above except that the minipumps contained sodium arsenate radiolabelled with carrier-free ^{74}As-arsenate purchased from Amersham-Searle. Animals were killed by CO_2 inhalation at specific times after terminating the heat exposure. Time of death was based on preliminary findings for animals killed 10 minutes, 1 hour, and 3 hours post-hyperthermia. maternal blood samples were obtained by cardiac puncture and placentas were removed by dissection. Weighed maternal blood and placenta samples were analyzed for their arsenic content by using a Beckman Gamma 5500. Total arsenic concentrations were calculated by comparing sample counts with those of an arsenate solution having the same ^{74}arsenic enrichment as that used in the minipumps.

Blood analyses

Creatinine assays were performed on plasma samples by using a colorimetric assay employing picric acid (Sigma Corp., Diagnostic Kit #555). Urea concentrations in maternal blood plasma were determined with a Beckman ASTRA-8 analyzer.

Body temperature measurements

Rectal temperatures (YSI-TeleThermometer) were determined for all animals prior to heat exposure. Pretreatment temperatures ranged from 36.8 to 37.1°C. Temperatures were recorded after 30 minutes of heat exposure, at the termination of the 50-minute exposure period and at 15 minutes and 30 minutes postexposure.

RESULTS

Data for teratogenic effects of continous exposure to arsenate and for the arsenate plus hyperthermia treatments are listed in Table 1. Heat-treated dams produced litters with a greater percent of malformed fetuses than did dams receiving no heat treatment. In both cases the statistical confidence level was greater than 99%.

Exposure to heat produced increases in the concentration of arsenic in maternal blood and placentas for hamsters receiving both arsenate dose regimens (see Figs. 1, 2). Maternal blood arsenic concentrations of heat-treated dams were near those of the arsenate dosed animals receiving no heat treatment 2 hours post-hyperthermia for hamsters exposed to the low-dose arsenate and 3 hours post heat treatment for hamsters exposed to the higher-dose regimen. The elevation in placental concentrations of arsenic was prolonged relative to maternal blood levels for both arsenate exposures. In addition, placental arsenic concentrations remained elevated for a longer period for the higher-arsenate-dose regimen.

Creatinine and urea concentrations in maternal plasmas of untreated, heat-treated, arsenate-treated, and arsenate-plus-heat-treated hamsters are given in Table 2.

Our data for body temperature changes of heat-treated hamsters showed a rise in their rectal temperatures from 37°C to 38°C over the first 30 minutes of heat exposure. At the termination of the heat treatment (50 min-

TABLE 1. The teratogenic response in hamsters following continuous exposure to arsenate via osmotic minipumps implanted on day 6 of gestation in combination with acute hyperthermia on the morning of day 8 of gestation

Concentration of arsenate in minipump (heat treatment)	No. of mothers	No. of gestation sacs	No. of resorptions (%)	Teratogenic response of surviving fetuses (%)	
				Normal	Malformed
0.321 M (no heat)	10	155	10 (6.4)	139 (95.8)	6 (4.2)
0.321 M (+ heat)	13	181	9 (5.0)	142 (82.0)	30 (18.0)
0.482 M (no heat)	10	151	13 (8.6)	127 (92.0)	11 (8.0)
0.482M (+ heat)	17	241	41 (17.0)	111 (60.0)	78 (39.0)

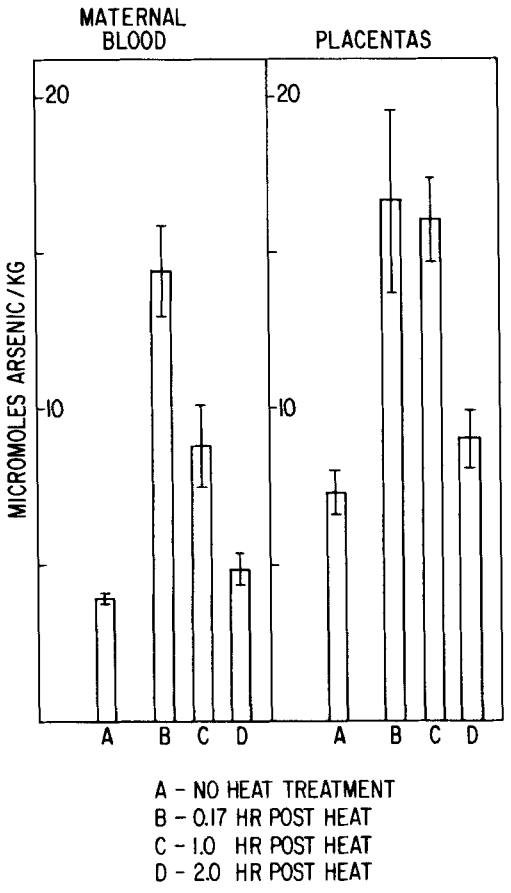

Fig. 1. Arsenic concentrations in the maternal blood and placentas of arsenate dosed hamster dams on the morning of day 8 of gestation. The dosing method and heat treatment are described in the text. Implanted minipumps contained 0.321 M sodium arsenate, radiolabelled with ^{74}arsenic. Vertical lines represent standard errors of means. Values are based on findings for four to six animals.

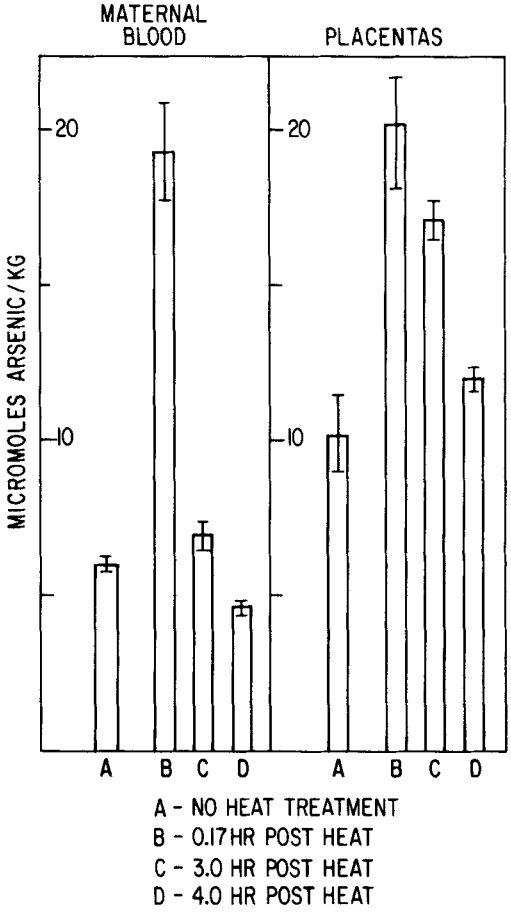

Fig. 2. Arsenic concentrations in the maternal blood and placentas of arsenate-dosed hamsters on the morning of day 8 of gestation. The dosing method and heat treatment are described in the text. Implanted minipumps contained 0.482 M sodium arsenate, radiolabelled with ^{74}arsenic. Vertical lines represent standard errors of means. Values are based on findings for four to six animals.

utes total) core temperatures were near 41°C. Fifteen minutes post heat, core temperatures were in the same range as those of hamsters not exposed to heat. Arsenate-dosed hamsters had the same core temperatures as nontreated animals.

DISCUSSION

Continuous exposure of hamster dams to arsenate during organogenesis produces a minimal and slightly greater than a minimal teratogenic response when minipumps are charged with 0.321 M arsenate and 0.482 M arsenate (see Table 1). The teratogenic data for the higher-dose regimen agree closely with findings reported earlier by ourselves (Ferm and Hanlon, '85). We consider the teratogenic responses reported here as real, albeit small, since the spontaneous incidence of malformations in our hamster model has remained unchanged at less than 0.1% since Ferm ('66) first reported this value. Heat treatment of hamster dams continuously dosed with arsenate causes a greater percent of living fetuses to be malformed for both dose regimens (see Table 1). Values of 18% malformation for the lower arsenate exposure and 39% malformation for the higher

TABLE 2. Creatinine and urea concentrations in the blood plasma of pregnant hamsters[1]

Treatment	No. of samples[2]	mg creatinine/dl	mg urea/dl
None	6	0.310 ± 0.011	16.3 ± 1.0
Acute hyperthermia	4	0.530 ± 0.039	20.0 ± 0.6
Arsenate exposure (0.482M arsenate in minipumps)	5	0.261 ± 0.005	16.0 ± 0.7
Arsenate + exposure + acute hyperthermia	6	0.495 ± 0.004	18.2 ± 1.2

[1]Data represent mean values ± standard errors. All blood samples were taken on the morning of day 8 of gestation.
[2]Samples were drawn from heat-treated dams 5–10 minutes post-hyperthermia.

arsenate exposure are statistically different from the values for the two arsenate-only treatments with $P < .01$ in both cases. Our present findings indicate that continuous exposure of hamster dams to arsenate in concert with an acute hyperthermia episode on day 8 of gestation results in a teratogenic response qualitatively similar to that found for acute arsenate dosing followed immediately by an acute hyperthermia episode on day 8 of gestation (Ferm and Kilham, '77).

What is the basis of this enhancement? The hyperthermia-induced rise in maternal blood arsenic and placental blood arsenic concentrations (see Figs. 1, 2) could well provide the answer. Although transient, the increased placental arsenic concentrations could be expected to provide increased exposure of embryos to arsenic during the critical phase of organogenesis. It would be difficult to obtain useful data directly from early day 8 embryos since the counting error would be very high due to their very small mass. We do know that the hamster placenta is permeable to arsenic during organogenesis. Embryos collected on day 9 of gestation contain radiolabelled arsenic following an acute dose of arsenate on the morning of day 8 of gestation (Hanlon and Ferm, '77).

At present we have no explanation for the reversible rise in the arsenic concentrations in maternal blood and placentas of the heat-treated hamsters. It cannot be accounted for by an increase in the pumping rate of the minipumps. The temperature dependency for pumping rates of Alzet osmotic minipumps has $Q_{10}=2.0$ (information provided by Alza Co., Palo Alto, California). Our calculations show that the temperature-dependent increase in the arsenate dose delivered accounts for less than 15% of the observed increases in arsenic concentrations in maternal blood and placentas of heat-treated dams. Part of the rise in arsenic concentration in maternal blood and placentas could be due to decreased kidney function precipitated by heat stress. A near twofold-greater plasma creatinine concentration and a more than 20% greater plasma urea concentration for maternal blood samples drawn immediately after terminating the heat treatment (see Table 2) could indicate a decreased glomerular filtration rate. The effect of the heat treatment on plasma creatinine and urea concentrations appears to be independent of continuous exposure to arsenate. Arsenate alone (at the higher-arsenate-exposure regimen) has no effect on plasma levels of these compounds.

An increased rate of protein catabolism in the heat-treated dams could also account for our creatinine and urea data, but there is no direct evidence that short-term stress produces this effect (Klosing, '85). Finally, some of the rise in arsenic concentration in placentas (and maternal blood) could be due to a general response to heat stress which causes the release of arsenic from unknown body stores, but we have no evidence of this.

We should point out that hyperthermia alone induces teratogenic lesions similar in nature to those associated with arsenate treatment in the hamster (Ferm and Kilham, '77). For this reason, we cannot state positively that the teratogenic response produced by arsenate/hyperthermia is due solely to increased arsenic concentrations at critical sites in the embryonic unit. However, our data provide support for this thesis.

Our findings for the hamster model suggest continued concern be shown for the human. The possibility that maternal hyperthermia could synergistically enhance the teratogenic potential of agents found in the diet, home environment, or workplace must be considered, particularly in view of the fact

that many women continue to work during early pregnancy. Likewise, endogenous hyperthermia (fever) during early human pregnancy could be a more significant risk factor for women exposed to environmental teratogens or who are receiving medications which possess teratogenic potential.

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