

Evaluation of the Reproductive and Developmental Safety of Cysteamine in the Rat: Effects on Female Reproduction and Early Embryonic Development

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ABSTRACT Cystinosis is an autosomal recessive metabolic disease in which the amino acid cystine accumulates in lysosomes due to a defect in lysosomal cystine transport. Cystinosis in infancy is associated with poor growth, muscle wastage, and death at about age 10 due to kidney failure. Treatment with cysteamine and kidney transplantation enables cystinotic girls to reach reproductive age and to be healthy enough to permit pregnancy. It is not known whether exposure to cysteamine will have adverse effects on reproduction in the human. It is also possible that some of the complications seen in cystinotic children could be avoided if a pregnant woman carrying a cystinotic fetus were given cysteamine. However, this treatment is not likely to occur until therapeutic exposures to cysteamine are judged to present no increased risk to the human fetus. As part of a larger investigation assessing the reproductive and developmental safety of cysteamine (as phosphocysteamine) using the rat, the two studies reported herein were performed. The first, a dose-finding study, led to the selection of 150 mg/kg/day as the highest dose of cysteamine used for the second and primary focus of this report. The second study involved the exposure of female rats to cysteamine from pre-mating through day 6.5 postconception and assessment of female fertility and early embryonic development. Cysteamine was administered orally in doses of 0, 37.5, 75, 100, or 150 mg/kg/day. There were no clinical signs of maternal toxicity during the exposures of 2 to 5 weeks before successful mating. Animals in the 150 mg/kg/day group experienced a nonsignificant decrease in body weight gain during pregnancy to day 6.5 postconception, a significant increase in liver and spleen weights, and a significant increase in days to coitus—suggesting that a low level of toxicity was manifested. However, there were no adverse effects on reproductive performance with respect to conception and early embryonic development. *Teratology* 58:88-95, 1998. © 1998 Wiley-Liss, Inc.

(Gahl, '86; Gahl et al., '88). First described in 1903 (Abderhalden, '03), the incidence is estimated (Gahl, '86) to be between 1 in 100,000 and 1 in 200,000 live births, but due to reasons explained below there are only about 350 individuals currently alive with cystinosis in the United States. Cystinosis in infancy is associated with renal Fanconi syndrome (impairment of proximal renal tubular resorption), poor growth, muscle wastage, and renal failure. These effects are caused by cellular death resulting from the excess storage of cystine in lysosomes within cells. Until the mid-1970's, 100% of children with cystinosis died by about age 10 due primarily to renal failure (Charnas et al., '94). Renal transplantation was initiated to extend the life of affected children, but children receiving kidney transplants suffered from complications accompanying the degeneration of other body tissues (Fink et al., '89; Schneider et al., '90; Theodoropoulos et al., '95; Charnas et al., '94). In 1994, the FDA approved the drug cysteamine (Cystagon[®], Mylan Pharmaceuticals, Morgantown, WV) for the treatment of cystinosis (Schneider, '95).

Cysteamine prevents the excess storage of cystine in lysosomes (Thoene et al., '76; Butler and Zatz, '84), and children with cystinosis must maintain treatment throughout their entire life (Reznik et al., '91; Markello et al., '93). Before cysteamine became available, the disease was associated with reproductive failure in males (Chik et al., '93), but not in females (Reiss et al., '88). Because a greater number of cystinotic girls will now reach reproductive age and have fewer symptoms of the disease, it is expected that some will become pregnant while taking cysteamine and thus expose their noncystinotic fetus to this drug.

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Cystinosis is an autosomal recessive metabolic disease in which the amino acid cystine accumulates in lysosomes due to a defect in lysosomal cystine transport

The most common identifiable cause of renal Fanconi syndrome is cystinosis (Gahl, '86). It is possible that the renal Fanconi syndrome in children with cystinosis could be avoided or reduced in severity if a pregnant woman carrying an affected fetus were given cysteamine (the fetus with cystinosis can be identified by measuring the cystine content of fetal cells obtained during amniocentesis or chorionic villus sampling). This treatment may reduce the need for renal transplantation in children with cystinosis. However, this treatment is not likely to occur until therapeutic exposures to cysteamine are judged to present no increased risk to the human fetus (W. Gahl, personal communication).

Cysteamine, a natural product of cells formed by the degradation of coenzyme A (Dupre et al., '70), has a chemical structure of $\text{NH}_2\text{—CH}_2\text{—CH}_2\text{—SH}$. Since the free thiol of cysteamine is a reactive reducing agent, there is a theoretical possibility that the drug will produce adverse effects on reproduction if given at a sufficiently high dose. Under laboratory conditions, exposure to cysteamine has been reported to have toxic effects *in vivo* and *in vitro*, including depletion of somatostatin, prolactin, and noradrenaline in the brain and peripheral tissues (Brown et al., '85; McComb et al., '85; Millard et al., '85; Ostenson and Efendic, '85; Vecsei and Widerlov, '90), production of Gomori-positive cytoplasmic inclusions indicating autophagocytized abnormal mitochondria in cultured astroglia (Mydlarski et al., '93; Schipper et al., '93; Brawer et al., '95), and perforating duodenal ulcers in rats (Selye and Szabo, '73; Szabo and Reichlin, '85). It is plausible biologically that cysteamine may result in reproductive and developmental toxicity.

Focusing on female reproduction and early embryonic development, extensive literature searches identified only three full publications which report on these issues after exposure *in utero* to potentially toxic doses of cysteamine in laboratory animals: Adams et al. ('61), Beliles and Scott ('67), and Manowska and Mazur ('88).

Adams et al. ('61) exposed pregnant rabbits to 70 to 160 mg of cysteamine hydrochloride per kg body weight on days 2 and 3, 3 and 4, or 4.5 and 5 by parenteral injection and found no adverse effect on blastocyst development, nidation, or further fetal development. The authors did not report how many animals were treated with cysteamine. The stage of development at which the exposure occurred was before implantation, and it was short; injections occurred on 2 consecutive days. Exposure during this time might be expected to result in embryonic death resulting in reduced implantation and, therefore, decreased litter size; but it would not be expected to affect organogenesis or fetal growth unless the treatment had continued adverse effects on the dam.

Beliles and Scott ('67) exposed male and female rats to 0, 75, and 375 mg cysteamine per kg body weight per day in their food for 70 days before mating, and then continued the exposure throughout gestation and lactation. Reduced litter size and postnatal growth and

survival was noted in the groups fed 375 mg/kg/day. An increase in malformations was not seen, but the data may not reflect accurately the incidence of adverse fetal outcome because natural delivery was permitted and, therefore, the dam may have consumed severely affected fetuses. Postnatal growth and survival were affected when the dam continued to consume cysteamine at an exposure of 375 mg/kg/day. The mechanism for these effects is not known.

Manowska and Mazur ('88) injected cysteamine intraperitoneally into mice at a dose of 80 mg/kg on the first day of gestation. They evaluated the fresh weight and glycogen content of fetal livers on the 19th day of pregnancy and found decreased liver weight and decreased glycogen content per gram of fresh tissue. The long-term consequences of these changes are not known.

It can be seen that the data concerning the reproductive and developmental toxicity of cysteamine are meager. The aim of this investigation was to determine whether cysteamine produces adverse effects on female reproduction or early embryonic development in the rat.

Cysteamine (as phosphocysteamine; Medea Research Laboratories, Port Jefferson Station, NY) was administered orally (described on the following page), as it is in the human. Because a pharmacokinetic comparison for cysteamine exposure to the developing conceptus of the human and the rat has not been performed, a simple mg of cysteamine per kg body weight per day was used as the basis for exposure as a best-available approximation.

The doses of cysteamine were multiples of a therapeutic dose in the human, 75 mg/kg/day. Because cysteamine can induce duodenal ulceration in the rat (Szabo, '78), which would adversely affect maternal health and thereby bias the results, the upper limit for the exposure was determined by direct experiment.

MATERIALS AND METHODS

Avoidance of duodenal ulceration

Female Wistar rats (Charles River Breeding Laboratories, Wilmington, MA), approximately 175 gm, were numbered and acclimated to the animal facility for 1 week in individual cages. Food and water were provided *ad libitum*. Each rat was observed for signs of ill health and body weight, and food consumption were determined daily.

Cysteamine was administered daily for 4 days between 8 and 9 a.m. by oral injection using a 76.2-mm curved feeding tube with a 3-mm ball tip. Repeated administration was selected to mirror the exposure paradigm in the subsequent experiment, and 4 days of exposures was selected based on the observation (Tanaka et al., '90) that macroscopic duodenal ulceration may be evident as early as 60 hr after dosing, but certainly by day 5 of cysteamine administration. The doses were 0, 75, 150, 225, 300, or 375 mg of cysteamine per kg body weight, with five animals in each group. The cysteamine was administered in a 10% (w/v) aque-

ous solution; the 0 mg/kg group received the volume of water equivalent to that in the highest dose group.

On day 5, the rats were sacrificed by cervical dislocation after induction of CO₂ narcosis. The duodenum was removed, flushed with saline, and placed in a petri dish containing Bouin's fixative. The specimen was opened using fine scissors and evaluated for macroscopic duodenal ulceration by examining the mucosal surface for damage with the aid of a dissection microscope, magnification $\times 10$ (Tanaka et al., '90). After fixation for 24 hr, the 30 specimens were processed for histological evaluation using standard methods. Histological evaluation was used to confirm or refute the presence of ulceration in each specimen, including those specimens where macroscopic ulceration was not evident.

Statistical analysis. Chi-square analysis was performed on the incidence of macroscopic duodenal ulceration. The numbers of animals in each group rendered a statistical power of 0.8 to detect differences between groups of $P < 0.05$; values given are mean \pm SD.

Female reproduction and early embryonic development

Female Wistar rats, approximately 175 g, were numbered and acclimated to the facility for 1 week in individual cages with a light cycle of 12 hr light/12 hr dark. Food and water were provided ad libitum. Each rat was weighed daily and observed for signs of ill health, and food consumption was determined daily.

During the 2-week predosing period, vaginal smears were obtained daily to document the duration of estrus for each rat. Vaginal smears were prepared from a sterile saline lavage and sprayed with CytoPrep Fixative (Fisher Scientific, Pittsburgh, PA). The specimens were stained using the Papanicolaou technique (Bancroft and Stevens '90), substituting sodium iodate for mercuric oxide and using commercially available stains (Fisher Scientific). The stained specimens were evaluated and the stage of the estrous cycle determined without knowledge of the treatment group. The length of the estrous cycle was defined as the days elapsed between subsequent observations of estrus.

At the end of the predosing period, oral administration of cysteamine was initiated. Cysteamine was then administered daily between 8 and 9 a.m. using a curved feeding tube with a ball tip. Evaluation of the results from the first experiment suggested that a trend of decreasing body weight began at a dose of 225 mg/kg/day (see below). The next lower dose, 150 mg/kg/day, was, therefore, selected as the highest dose in this phase of the study in order to reduce adverse effects on the dams during the extended period of treatment with cysteamine. The daily doses were 0, 37.5, 75, 100, or 150 mg of cysteamine per kg body, with 15 to 20 animals in each group. The cysteamine was administered in a 10% (w/v) aqueous solution; the 0 mg/kg group received the volume of water equivalent to that in the highest dose group. Daily administration of cysteamine was continued through the next 2 weeks, the mating period

of up to 3 weeks, and during pregnancy until the rats were sacrificed on day 6.5 postconception.

During the 2-week period of dosing nonpregnant rats, vaginal smears were obtained daily as above, to document possible changes in estrous duration. The female rats were then placed overnight (i.e., 4 p.m. until 8 a.m.) in cages with male rats of the same strain. Sperm found in the vaginal lavage was used to define 0.5 days postconception. Sperm-negative females were placed in individual cages until 4 p.m., at which time they were again placed overnight in cages with males. The procedure was repeated for a maximum of 3 weeks or until sperm were found in the vaginal lavage. Females found to be sperm-negative at the end of 3 weeks of attempted mating were sacrificed.

Sperm-positive females were housed individually. These females were sacrificed on day 6.5. The uterus and attached ovaries were removed, immersed in 10% ammonium sulfide for 10 min in a fume hood, and then inspected for the number of corpora lutea, the number of implantations, and the number of implantations which were resorbing.

Organ weights for the heart, liver, spleen, left kidney, and right kidney were obtained at the time of sacrifice on day 6.5 of pregnancy from five to eight randomly selected rats from each of the treatment groups. In addition, specimens from the kidney, spleen, liver, heart, and duodenum were placed in fixative and processed for histological examination using standard procedures.

Statistical analyses. All dependent variables were on the interval level of measurement. Dependent variables measured on multiple occasions were analyzed using repeated measures Analysis of Variance (ANOVAs) (e.g., daily food consumption and daily body weight). Significant findings were followed up by dependent *t*-tests corrected for multiple contrasts by the Bonferroni adjustment (main effects for time) or one-way ANOVAs, with the post hoc, experimentwise error control through the Tukey adjustment.

Dependent variables measured on one occasion were analyzed using one-way ANOVAs, with post hoc comparisons completed through the Tukey correction.

RESULTS

Avoidance of duodenal ulceration

One rat in the group receiving 375 mg/kg/day was removed from the study due to injury unrelated to the exposure to cysteamine.

At the time of sacrifice on day 5, the animals in groups receiving 0 through 225 mg/kg appeared normal and healthy. In the 300 mg/kg group, one animal had a small amount of nasolacrimal hemorrhage around both eyes, and two had similar hemorrhage surrounding the nares which were not evident on the previous day. Minor nasolacrimal hemorrhage was observed around the eyes of one rat in the 375 mg/kg group. The values for body weight and food consumption for the 375 mg/kg/day group were significantly less ($P < .05$) than

TABLE 1. Body weight (g) of nonpregnant female rats administered daily oral doses of cysteamine*

Day of administration	Cysteamine administered, mg/kg/day					
	0	75	150	225	300	375
1	198 ± 10	194 ± 5	192 ± 11	196 ± 9	201 ± 5	200 ± 6
2	193 ± 11	196 ± 3	192 ± 13	194 ± 7	195 ± 9	196 ± 4
3	196 ± 10	203 ± 6	198 ± 17	196 ± 4	193 ± 11	196 ± 10
4	197 ± 12	204 ± 5	200 ± 13	195 ± 7	184 ± 14	182 ± 9
5	204 ± 16	199 ± 6	199 ± 10	193 ± 9	178 ± 15**	178 ± 8**

*Values are mean ± SD—four animals in 375 mg/kg/day group and five animals in all other groups.

**Significantly different from the value for the 0 mg/kg/day group, $P < .05$.

TABLE 2. Food consumption (g) by nonpregnant female rats administered daily oral doses of cysteamine*

Day of administration	Cysteamine administered, mg/kg/day					
	0	75	150	225	300	375
1	—	—	—	—	—	—
2	10 ± 4	18 ± 4	15 ± 6	13 ± 3	10 ± 5	12 ± 3
3	17 ± 4	22 ± 3	21 ± 5	18 ± 3	10 ± 5	11 ± 2
4	17 ± 4	21 ± 2	20 ± 1	16 ± 4	6 ± 7**	2 ± 3**
5	20 ± 5	17 ± 2	20 ± 4	16 ± 6	10 ± 5**	8 ± 8**

*Values are mean ± SD food consumption over the previous 24 hr—four animals in 375 mg/kg/day group and five animals in all other groups.

**Significantly different from the value for the 0 mg/kg/day group, $P < .05$.

those for the 0 mg/kg/day group on days 4 and 5 (Tables 1 and 2). Body weight and food consumption were significantly decreased for the 300 mg/kg/day group on day 5 only.

The duodenum was unremarkable macroscopically in the females in the 0 through 300 mg/kg treatment groups. In the 375 mg/kg group, two specimens were visually inflamed and macroscopic examination revealed small focal points of what appeared to be hemorrhage in the mucosa. The incidence of these sites was approximately two per square centimeter, and each site was approximately 1 mm in diameter. The remaining two specimens in this group were unremarkable.

Histological observations were performed without knowledge of exposure group. Histological examination of the duodenum specimens employed Mowry's staining for acid mucopolysaccharides and Hematoxylin and Eosin (H & E) staining for general cytological differentiation. Mowry's staining revealed normal distribution and number of goblet cells in all specimens. Increased lymphocyte proliferation in the lamina propria was observed in two of four in the 375 mg/kg/day group. The crypts of Lieberkuhn appeared unaltered. Other aspects of the morphology of the villi were within normal variation.

H & E staining revealed early focal inflammation, increased lymphocyte proliferation, and some congestion in the mucosa of the two specimens from the 375 mg/kg/day group in which alterations were observed macroscopically. No hemorrhage was found. No ulceration was found. All other cell layers in these two specimens were unremarkable. No alterations were

observed in the remaining specimens in this or other groups.

Female reproduction and early embryonic development

There was a significant main effect for time ($F = 2,671.25$, $df[4,5]$, $P < .001$) for female body weight (Table 3). No dose-associated difference was detected ($F = 1.45$, $df[4,5]$, $P = .226$), nor was the group × time comparison significant ($F = .92$, $df[4,5]$, $P = .557$). Thus, the body weight of rats at the final day of each experimental period in all dose groups increased in a similar manner across the time period of the study. Nasolacrimal hemorrhage was observed rarely and inconsistently.

A significant group × time interaction ($F = 1.82$, $df[4,20]$, $P < .017$) was found for food consumption (Table 4). Post hoc analyses showed a difference between the 0 mg/kg/day group and the 75, 100, and 150 mg/kg/day groups only on the day before administration of cysteamine was initiated, but not at any other time in this study. We, therefore, concluded that the administration of cysteamine had no effect on food consumption during the prepregnancy period and up to day 6.5 of pregnancy.

There were no observed changes in the average weight of the heart and kidneys, but the spleen and liver were significantly increased ($P < .05$) in the 150 mg/kg/day group (Table 5). Histological examinations of kidney, spleen, liver, heart, and duodenum biopsies revealed no alterations.

TABLE 3. Body weight (g) of female rats during the three periods of the study*

Period of study, days	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Prior to administration of cysteamine and not pregnant					
1	207 ± 14 (20)	207 ± 18 (20)	208 ± 15 (20)	200 ± 27 (15)	202 ± 15 (20)
2	205 ± 15 (20)	209 ± 15 (20)	208 ± 15 (20)	202 ± 20 (15)	203 ± 12 (20)
3	210 ± 14 (20)	211 ± 16 (20)	212 ± 15 (20)	202 ± 17 (15)	204 ± 10 (20)
4	211 ± 12 (20)	214 ± 16 (20)	214 ± 14 (20)	205 ± 16 (15)	211 ± 10 (20)
5	214 ± 14 (20)	215 ± 16 (20)	213 ± 16 (20)	208 ± 12 (15)	212 ± 11 (20)
6	214 ± 15 (20)	216 ± 15 (20)	216 ± 16 (20)	212 ± 13 (15)	217 ± 12 (20)
7	219 ± 15 (20)	219 ± 19 (20)	217 ± 18 (20)	212 ± 10 (15)	215 ± 11 (20)
8	218 ± 13 (20)	220 ± 17 (20)	221 ± 13 (20)	214 ± 12 (15)	218 ± 11 (20)
9	218 ± 14 (20)	222 ± 16 (20)	220 ± 15 (20)	217 ± 14 (15)	221 ± 12 (20)
10	219 ± 14 (20)	222 ± 16 (20)	219 ± 17 (20)	220 ± 10 (15)	223 ± 11 (20)
11	223 ± 15 (20)	225 ± 16 (20)	223 ± 14 (20)	221 ± 12 (15)	222 ± 12 (20)
12	225 ± 13 (20)	226 ± 19 (20)	225 ± 14 (20)	224 ± 11 (15)	225 ± 11 (20)
13	227 ± 17 (20)	228 ± 17 (20)	229 ± 16 (20)	230 ± 13 (15)	231 ± 13 (20)
14	228 ± 16 (20)	231 ± 17 (20)	231 ± 15 (20)	230 ± 12 (15)	232 ± 13 (20)
Administration of cysteamine and not pregnant					
1	229 ± 16 (20)	232 ± 16 (20)	230 ± 15 (20)	228 ± 11 (15)	228 ± 12 (20)
2	229 ± 15 (20)	231 ± 16 (20)	230 ± 15 (20)	228 ± 10 (15)	229 ± 11 (20)
3	230 ± 16 (20)	230 ± 17 (20)	228 ± 15 (20)	227 ± 12 (15)	230 ± 12 (20)
4	230 ± 16 (20)	234 ± 16 (20)	232 ± 14 (20)	227 ± 9 (15)	229 ± 12 (20)
5	233 ± 16 (20)	235 ± 17 (20)	235 ± 13 (20)	231 ± 9 (15)	231 ± 12 (20)
6	237 ± 17 (20)	241 ± 20 (20)	240 ± 13 (20)	234 ± 9 (15)	230 ± 15 (20)
7	241 ± 19 (20)	243 ± 18 (20)	240 ± 14 (20)	237 ± 10 (15)	232 ± 15 (20)
8	237 ± 17 (20)	240 ± 17 (20)	238 ± 13 (20)	236 ± 10 (15)	231 ± 14 (20)
9	238 ± 17 (20)	242 ± 18 (20)	240 ± 13 (20)	238 ± 9 (15)	231 ± 14 (20)
10	240 ± 17 (20)	242 ± 18 (20)	240 ± 12 (20)	238 ± 10 (15)	232 ± 14 (20)
11	241 ± 18 (20)	243 ± 18 (20)	240 ± 13 (20)	239 ± 9 (15)	233 ± 14 (20)
12	240 ± 18 (20)	242 ± 17 (20)	239 ± 13 (20)	239 ± 10 (15)	234 ± 14 (20)
13	245 ± 18 (20)	246 ± 19 (20)	246 ± 14 (20)	242 ± 11 (15)	238 ± 16 (20)
14	248 ± 19 (20)	248 ± 20 (20)	248 ± 12 (20)	241 ± 10 (15)	238 ± 16 (20)
Administration of cysteamine and pregnant					
0.5	249 ± 16 (19)	251 ± 23 (20)	250 ± 14 (18)	244 ± 9 (11)	241 ± 15 (20)
1.5	257 ± 22 (19)	253 ± 23 (20)	253 ± 16 (18)	243 ± 11 (11)	241 ± 16 (20)
2.5	260 ± 22 (19)	257 ± 24 (20)	257 ± 15 (18)	251 ± 12 (11)	246 ± 16 (20)
3.5	264 ± 21 (19)	262 ± 22 (20)	259 ± 15 (18)	252 ± 10 (11)	246 ± 16 (20)
4.5	269 ± 24 (19)	264 ± 22 (20)	263 ± 14 (18)	254 ± 10 (11)	248 ± 16 (20)
5.5	270 ± 22 (19)	267 ± 22 (20)	266 ± 14 (18)	255 ± 11 (11)	249 ± 16 (20)
6.5	274 ± 22 (19)	268 ± 21 (20)	267 ± 14 (18)	256 ± 14 (11)	250 ± 17 (20)

*Values are mean ± SD and, in parentheses, the number of female rats.

The duration of the estrous cycle, days to coitus, and number of corpora lutea were used to evaluate the effect of cysteamine on the reproductive potential in the female. The results are presented in Table 6. There was no significant difference ($P > .05$) in the duration of the estrous cycle before and during administration of cysteamine—although there was a trend towards increased duration of diestrus. There was also no significant difference ($P > .05$) in the number of corpora lutea. In contrast, there was a significant increase ($P < .02$) in the number of days to coitus for the 150 mg/kg/day group, but there was no significant difference ($P > .05$) in the number of female rats who became pregnant within the 3-week period in each treatment group.

Reproductive success was evaluated using the number of implantation sites and the number of failed conceptions (Table 6). The number of failed conceptions was calculated as the difference between the number of

corpora lutea and the number of implantation sites. There was no treatment-related effect on the number of implantation sites or the number of failed conceptions ($P > .05$).

DISCUSSION

This study focused on the question of whether cysteamine affects female reproduction and early embryonic development using doses which did not produce duodenal ulceration in the female rat. Analysis of the results of the first experiment revealed no statistically significant differences between the 0 mg/kg/day group and the 75, 150, or 225 mg/kg/day treatment groups. However, a nonstatistically significant trend in body weight decrease was judged to be present in the 225 mg/kg/day group, suggesting that this dose was likely to result in adverse effects in the dams with an extended duration

TABLE 4. Food consumption (g) by female rats during the three periods of the study*

Period of study, days	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Prior to administration of cysteamine and not pregnant					
2	19 ± 5 (20)	21 ± 4 (20)	19 ± 4 (20)	19 ± 3 (15)	20 ± 4 (20)
3	20 ± 5 (20)	20 ± 4 (20)	20 ± 6 (20)	19 ± 4 (15)	20 ± 5 (20)
4	20 ± 4 (20)	22 ± 4 (20)	22 ± 4 (20)	21 ± 5 (15)	24 ± 2 (20)
5	21 ± 3 (20)	20 ± 5 (20)	20 ± 5 (20)	21 ± 3 (15)	23 ± 3 (20)
6	18 ± 4 (20)	20 ± 4 (20)	20 ± 4 (20)	21 ± 4 (15)	23 ± 4 (20)
7	19 ± 4 (20)	22 ± 4 (20)	20 ± 5 (20)	18 ± 6 (15)	20 ± 5 (20)
8	21 ± 4 (20)	22 ± 4 (20)	25 ± 3 (20)	23 ± 6 (15)	24 ± 3 (20)
9	20 ± 4 (20)	21 ± 4 (20)	20 ± 4 (20)	23 ± 5 (15)	23 ± 5 (20)
10	20 ± 5 (20)	22 ± 4 (20)	21 ± 5 (20)	23 ± 3 (15)	23 ± 4 (20)
11	21 ± 4 (20)	22 ± 4 (20)	22 ± 4 (20)	21 ± 5 (15)	22 ± 4 (20)
12	22 ± 3 (20)	22 ± 6 (20)	23 ± 3 (20)	22 ± 3 (15)	23 ± 4 (20)
13	21 ± 5 (20)	21 ± 4 (20)	21 ± 5 (20)	23 ± 4 (15)	24 ± 4 (20)
14	18 ± 4 (20)	21 ± 4 (20)	22 ± 3 (20)**	23 ± 4 (15)**	24 ± 4 (20)**
Administration of cysteamine and not pregnant					
2	21 ± 4 (20)	19 ± 3 (20)	18 ± 4 (20)	19 ± 5 (15)	19 ± 5 (20)
3	20 ± 5 (20)	19 ± 4 (20)	18 ± 6 (20)	18 ± 5 (15)	20 ± 5 (20)
4	19 ± 4 (20)	22 ± 4 (20)	22 ± 4 (20)	20 ± 6 (15)	20 ± 5 (20)
5	21 ± 4 (20)	22 ± 4 (20)	22 ± 3 (20)	21 ± 5 (15)	21 ± 5 (20)
6	21 ± 3 (20)	22 ± 5 (20)	21 ± 4 (20)	23 ± 4 (15)	21 ± 10 (20)
7	22 ± 4 (20)	23 ± 4 (20)	23 ± 5 (20)	24 ± 4 (15)	21 ± 6 (20)
8	20 ± 4 (20)	23 ± 2 (20)	23 ± 3 (20)	25 ± 3 (15)	23 ± 6 (20)
9	20 ± 3 (20)	22 ± 4 (20)	22 ± 4 (20)	24 ± 4 (15)	22 ± 5 (20)
10	21 ± 4 (20)	22 ± 4 (20)	22 ± 3 (20)	23 ± 4 (15)	23 ± 4 (20)
11	21 ± 4 (20)	22 ± 4 (20)	20 ± 3 (20)	24 ± 3 (15)	23 ± 4 (20)
12	20 ± 5 (20)	22 ± 3 (20)	21 ± 4 (20)	23 ± 2 (15)	23 ± 4 (20)
13	21 ± 3 (20)	22 ± 4 (20)	22 ± 3 (20)	21 ± 4 (15)	22 ± 3 (20)
14	23 ± 5 (20)	23 ± 4 (20)	24 ± 4 (20)	26 ± 6 (15)	23 ± 4 (20)
Administration of cysteamine and pregnant					
1.5	24 ± 4 (19)	23 ± 5 (20)	25 ± 4 (18)	21 ± 6 (11)	24 ± 7 (20)
2.5	25 ± 3 (19)	25 ± 5 (20)	25 ± 2 (18)	26 ± 4 (11)	26 ± 3 (20)
3.5	25 ± 4 (19)	25 ± 4 (20)	25 ± 3 (18)	26 ± 4 (11)	24 ± 4 (20)
4.5	27 ± 4 (19)	25 ± 5 (20)	25 ± 3 (18)	26 ± 3 (11)	26 ± 3 (20)
5.5	27 ± 4 (19)	27 ± 5 (20)	27 ± 3 (18)	27 ± 4 (11)	25 ± 4 (20)
6.5	27 ± 4 (19)	26 ± 4 (20)	27 ± 4 (18)	26 ± 4 (11)	25 ± 4 (20)

*Values are mean ± SD food consumption over the previous 24 hr and, in parentheses, the number of female rats.

**Significantly different from the value for the 0 mg/kg/day group, $P < .05$.

TABLE 5. Organ weight (g) in rats on day 6.5 of pregnancy after 5–7 weeks of daily doses of cysteamine*

Organ	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Total body	283 ± 21	285 ± 14	270 ± 19	253 ± 20	258 ± 25
Heart	0.823 ± 0.082	0.937 ± 0.036	0.850 ± 0.079	0.884 ± 0.056	0.934 ± 0.094
Left kidney	0.885 ± 0.023	0.937 ± 0.030	0.930 ± 0.086	0.953 ± 0.074	1.072 ± 0.121
Right kidney	0.919 ± 0.032	0.945 ± 0.050	0.954 ± 0.111	0.951 ± 0.075	1.083 ± 0.126
Liver	10.5 ± 1.1	11.5 ± 1.0	11.3 ± 1.0	10.6 ± 1.5	12.4 ± 1.9**
Spleen	0.764 ± 0.076	0.805 ± 0.089	0.753 ± 0.149	0.760 ± 0.100	1.065 ± 0.150**

*Values are mean ± SD, five to eight animals per group.

**Significantly different from the value for the 0 mg/kg/day group. $P < .05$.

of treatment. Therefore, to avoid adverse effects on the dam in the subsequent experiment, 150 mg/kg/day was selected as an appropriate maximum dose.

The results reported herein on exposures extending from pre mating to 6.5 days postconception provide information on the reproductive risk of cysteamine at a very vulnerable stage of pregnancy. A substantial por-

tion of human embryonic loss occurs early in pregnancy—possibly as many as one-half of conceptions in the human are lost before the pregnancy is clinically recognizable (Hertig, '67; Robert and Lowe, '75; Klein and Stein, '85). In rodents, it has been shown that exposure of the embryo to a known developmental toxicant during the very early stages of embryonic

TABLE 6. Female reproduction and early embryonic development in rats administered daily doses of cysteamine from pre mating to day 6.5 of pregnancy*

Parameters of reproduction and early development	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Duration of estrous, days					
Prior to administration	4.2 ± 0.7 (20)	4.1 ± 0.5 (20)	4.4 ± 0.8 (20)	4.5 ± 1.0 (15)	4.5 ± 1.0 (20)
During administration	4.4 ± 0.6 (20)	4.1 ± 0.2 (20)	4.8 ± 1.7 (20)	5.3 ± 1.4 (15)	5.5 ± 2.7 (20)
Days to coitus	2.3 ± 1.6 (19)	3.1 ± 2.2 (20)	2.7 ± 2.8 (18)	4.1 ± 3.7 (13)	5.4 ± 4.1** (20)
Rats pregnant at end of 3 weeks	19 (20)	20 (20)	18 (20)	11 (15)	20 (20)
Corpora lutea per litter	14.9 ± 2.2 (19)	16.0 ± 2.1 (20)	15.4 ± 1.6 (18)	15.1 ± 2.4 (11)	14.9 ± 2.0 (20)
Implantation sites per litter	12.9 ± 3.8 (19)	10.0 ± 6.8 (20)	10.1 ± 6.3 (18)	11.1 ± 5.3 (11)	13.2 ± 2.0 (20)
Failed conceptions per litter	2.1 ± 3.1 (19)	6.0 ± 6.9 (20)	5.3 ± 5.7 (18)	4.0 ± 4.9 (11)	1.8 ± 1.1 (20)

*Values are mean ± SD and, in parentheses, the number of female rats.

**Significantly different from the value for the 0 mg/kg/day group, $P < .05$.

development, from fertilization through early postimplantation, is most likely to result in embryonic death (Brent and Bolden '68; Russell and Russell '50, '54). This investigation was not designed to determine the reason for a difference in the number of corpora lutea and the number of implantation sites. Since the mechanism leading to a difference between the ova produced and the implantation sites present on day 6.5 of pregnancy is not known, the difference was considered to be a loss of a potential embryo and combined with the recognized resorptions to yield an estimate of failed conceptions. It is recognized that this decision will overestimate any adverse effect that cysteamine may have on preimplantation development. Even so, it can be seen from Table 6 that cysteamine had no demonstrable effect on the number of failed conceptions.

During the period of treatment before successful mating, there were no clinical signs of toxicity in the dams. However, the dams in the highest exposure group experienced a statistically insignificant decrease in body weight gain during pregnancy, a significant increase in the weight of the liver and the spleen, and a significant increase in the days to coitus. Although these findings suggest that a low level of toxicity was manifested, there were no adverse effects on reproductive performance with respect to conception and early embryonic development.

The dose-response relationship for reproductive and developmental toxicity determined in a laboratory animal cannot be applied directly to the human condition with certainty if there is no information concerning adverse effects on the developing human. Nevertheless, the results of this study will assist in the evaluation of the long-term quality of life for children with cystinosis who are taking cysteamine, and the potential for adverse effects on reproduction in these patients.

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