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Calcium Homeostasis in Pregnant Rats Treated with Ethylene Glycol Monomethyl Ether (EGME)[1]

MARK TORAASON, RICHARD W. NIEMEIER, AND BRYAN D. HARDIN

U.S. Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226

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Calcium Homeostasis in Pregnant Rats Treated with Ethylene Glycol Monomethyl Ether (EGME). TORAASON, M., NIEMEIER, R. W., AND HARDIN, B. D. (1986). *Toxicol. Appl. Pharmacol.* **86,** 197–203. The industrial solvent ethylene glycol monomethyl ether (EGME) is a known teratogen that has been reported to alter calcium metabolism in guinea pigs during chronic exposure. Because of the tremendous demand of reproduction on maternal calcium stores, the effects of EGME on calcium and vitamin D metabolism during gestation were examined. Timed pregnant rats were treated by gavage with 0, 50, or 100 mg/kg EGME in 10 ml/kg distilled water on Days 9–15 of gestation (sperm = Day 1) and examined on Days 16 and 21. Virgin rats were treated for 7 days with 0 or 100 mg/kg EGME and examined 5 days later. EGME exposure did not affect body or kidney weight in virgin or pregnant rats, but liver weight was reduced in near-term pregnant rats treated with 100 mg/kg EGME. EGME (50 mg/kg) reduced litter size and fetal body weight and caused a significant number of live fetuses to have visceral abnormalities. EGME (100 mg/kg) caused all fetuses to be resorbed. In nonpregnant rats, 100 mg/kg did not affect serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), 25-hydroxyvitamin D, ionic calcium, total calcium, or parathyroid hormone. EGME appeared to have a dose-dependent effect on calcium and vitamin D metabolism during gestation. On Day 21 of gestation, total calcium and ionic calcium were increased and $1,25(OH)_2D_3$ was reduced in rats treated with EGME compared with nontreated controls. However, significant alterations in calcium homeostasis were evident only in dams that completely resorbed their litters. The changes in calcium and vitamin D metabolism during gestation appear to be secondary to the EGME-induced loss of litters.

Ethylene glycol monomethyl ether (EGME), also known as 2-methoxyethanol, has a wide variety of industrial applications. EGME is used as a solvent in lacquers, enamels, varnishes, inks, and dyes and as an anti-icing additive in fuels and other fluids (NIOSH, 1983). The toxicity of EGME has generated considerable interest lately because of its action as a potent developmental toxin and teratogen (Nagano *et al.*, 1981; Hardin, 1983; NIOSH, 1983; Nelson *et al.*, 1984; Nelson and Brightwell, 1984; Toraason *et al.*, 1985). In addition to its developmental toxicity, EGME acts on the central nervous, renal, and hematopoietic systems (Hardin, 1983). A recent report indicates that EGME may also interfere with calcium metabolism. Hobson *et al.* (1984) found that chronic treatment of guinea pigs with EGME markedly increased urinary excretion of calcium in the absence of any indication of renal damage. The possible action of EGME on calcium metabolism is of special concern during development because

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of the dynamic state of maternal calcium metabolism during the reproductive cycle.

During gestation in the rat, the rapid growth of the fetuses exerts a demand on maternal calcium stores, causing a significant reduction in serum calcium concentration (Halloran *et al.*, 1979). The reduced serum calcium stimulates release of parathyroid hormone (PTH) (Garabedian *et al.*, 1971), which acts in concert with reduced serum calcium to stimulate renal hydroxylation of 25-hydroxyvitamin D_3 ($25(OH)D_3$) to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Boass *et al.*, 1977; Pike *et al.*, 1979; Halloran *et al.*, 1979). This conversion leads to reduced concentrations of serum $25(OH)D_3$ and increased concentrations of $1,25(OH)_2D_3$ (Boass *et al.*, 1977; Pike *et al.*, 1979; Halloran *et al.*, 1979). The increased $1,25(OH)_2D_3$ acts to restore serum calcium by stimulating bone resorption, renal reabsorption, and intestinal absorption of calcium (Halloran and DeLuca, 1980a; Toraason, 1983).

The possible effect of EGME on calcium metabolism in guinea pigs, as well as its action as a developmental toxin, stimulated this study of maternal calcium homeostasis in rats treated with embryotoxic doses of EGME. It was hypothesized that the embryotoxic effects of EGME were associated with altered maternal calcium homeostasis. Calcium homeostasis was monitored by measuring serum concentrations of total calcium, ionic calcium, PTH, $25(OH)D_3$, and $1,25(OH)_2D_3$.

METHODS

Animals. Time-pregnant (sperm = Day 1) and virgin Sprague–Dawley rats were obtained from Charles River Breeding Labs, Wilmington, Massachusetts. Rats were housed individually in wire-mesh cages, and fed Purina rat chow and tap water *ad libitum*. Rats were maintained on a 12-hr light/dark cycle at 23 ± 2°C and 55 ± 15% relative humidity.

Treatment and termination. The day after arrival, pregnant rats were randomly assigned to two groups: those to be killed on gestation Day 16 (n = 36) and those to be killed on gestation Day 21 (n = 42). Each of these two groups was equally divided into three EGME treatment groups. Pregnant dams were treated by gavage with 0, 50, or 100 mg/kg EGME in 10 ml/kg distilled water on Days 9–15 of gestation. Virgin rats (n = 16) were divided into two EGME treatment groups, and were treated with 0 or 100 mg/kg on the same days as the pregnant dams. Virgin rats were killed 5 days after the last day of exposure, which corresponded to gestation Day 21 in the pregnant rats. The groups of pregnant rats were larger than the groups of virgin rats to permit the exclusion of dams found to have no implantation sites at the time of sacrifice. Certified-grade EGME was obtained from Fisher Scientific (Cat. No. E-182).

Rats were anesthetized with 40 mg/kg pentobarbital and blood was obtained by cardiac puncture. Uterus, liver, and kidney weights were recorded for all rats. For Day 21 pregnant dams, the number of implants and live fetuses, and fetal weights also were recorded. Fetuses were preserved intact in Bouin's solution and examined without knowledge of treatment or grouping for visceral abnormalities by Dr. Phillip T. Goad, Intox Laboratories, Inc. (Redfield, Ariz.) using the Wilson technique (Wilson, 1965).

Serum analysis. Analysis was performed using serum samples from six rats in each group. After the nonpregnant rats were excluded from the groups of pregnant rats, the heaviest and lightest dams were systematically excluded to reduce each group to six rats. Serum samples were assayed by Roche Biomedical Laboratories, Inc. (Burlington, N.C.). Total calcium was determined by atomic absorption. Ionic calcium was determined by ion-selective electrode. $1,25(OH)_2D_3$ and $25(OH)D_3$ were determined by a competitive protein-binding assay. PTH was determined using Immuno Nuclear Corporation's C-terminal PTH assay kit.

Statistical analysis. ANOVA and Duncan's multiple-range test were used to assess group differences for organ weights, body weights, and serum concentrations of calcium, vitamin D metabolites, and PTH. The number of litters in the 50-mg/kg EGME group having one or more fetuses with visceral malformations was compared with that in the control group using Fisher's exact test.

RESULTS

Maternal Toxicity

In virgin rats (data not shown) and rats killed on gestation Day 16 (Table 1), no significant changes were observed in body or organ weights. On gestation Day 21, gravid uterus weight was slightly reduced in rats treated with 50 mg/kg EGME and significantly reduced in rats treated with 100 mg/kg

TABLE 1

MATERNAL BODY AND ORGAN WEIGHTS AND REPRODUCTIVE OUTCOME IN EGME-TREATED AND CONTROL RATS

	EGME (mg/kg)		
	0	50	100
Dams sacrificed day 16			
Number pregnant	12	12	14
Maternal weight minus gravid uterus weight (g)	248 ± 27	255 ± 25	249 ± 21
Gravid uterus weight (g)	13 ± 3	10 ± 6	8 ± 5
Maternal kidney weight (g)	1.9 ± 2	1.8 ± 2	1.8 ± 2
Maternal liver weight (g)	12.5 ± 1.4	12.0 ± 1.7	12.0 ± 1.4
Implants/litter	12.8 ± 2.5	9.1 ± 4.1*	12.2 ± 4.1
Dams sacrificed day 21			
Number pregnant	14	14	13
Maternal weight minus gravid uterus weight (g)	281 ± 25	278 ± 30	267 ± 21
Gravid uterus weight (g)	65 ± 12	49 ± 16	6 ± 4*
Maternal kidney weight (g)	1.8 ± 2	1.9 ± 2	1.8 ± 2
Maternal liver weight (g)	15.6 ± 2.1	16.0 ± 1.7	12.9 ± 2.1*
Implants/litter	13.1 ± 1.1	11.5 ± 3.2	12.3 ± 2.1
Live fetuses/litter	12.4 ± 1.4	9.8 ± 3.1*	0
Percentage live fetuses (mean of litter mean %)	94 ± 7	86 ± 15*	0
Fetal weight (g)	3.5 ± 0.6	2.6 ± 0.6*	

Note. Weight values are means ± SD.

* Significantly different from control, $p < 0.05$.

EGME. Also in the 100-mg/kg EGME group, liver weight was significantly reduced below nontreated control dams.

Reproductive Outcome

In dams killed on Day 16, there was an unexpected significant reduction in implants in the dams treated with 50 mg/kg EGME. Three dams in this group had implants in only one horn of their uterus. This is not likely related to exposure since EGME treatment began after embryos were implanted, and since the effect was not evident in any other treatment group on Days 16 or 21. In dams terminated on gestation Day 21, EGME had a dose-dependent effect on reproductive outcome. There were no live fetuses in the 100-mg/kg EGME group. In the 50-mg/kg EGME group, the number of live fetuses and the percentage of live fetuses were significantly reduced as was fetal body weight (Table 1). There was also a significant increase in the percentage of malformed fetuses in the 50-mg/kg EGME group (Table 2); 93% of the malformations were cardiovascular.

Calcium Metabolism

The normal adjustments in calcium homeostasis during pregnancy can be seen by comparing the virgin and pregnant rats treated with 0 mg/kg EGME. Between Days 16 and 21 of gestation, total calcium concentration decreased significantly (Fig. 1). There was a corresponding drop in ionic calcium

TABLE 2

INCIDENCE OF FETAL MALFORMATIONS IN LITTERS FROM EGME-TREATED AND CONTROL RATS

	EGME (mg/kg)	
	0	50
Litters/fetuses	14 (173)	14 (137)
Cardiovascular		
Double aortic arch	0	9 (18)
Right aortic arch	0	9 (17)
Right ductus arteriosis	0	6 (11)
Ventricular septal defect	1 (1)	12 (54)
Total cardiovascular	1 (1)	14 (71)*
Urinary		
Hydronephrosis	3 (3)	4 (7)
Hydroureter	2 (2)	0
Missing bladder	0	1 (1)
Other		
Cleft palate	0	1 (1)
Tracheo-esophageal transposition	0	2 (3)
Total malformations (litters/fetuses)	4 (7)	14 (76)*
Percentage abnormal (mean of litter mean % ± SD)	4 ± 2	58 ± 8*

* Significantly different from control, $p < 0.0001$.

concentration (Fig. 2), although the decrease was not statistically significant. Coinciding with the decreased serum calcium was a significant decrease in $25(OH)D_3$ (Fig. 3) and a significant increase in $1,25(OH)_2D_3$ (Fig. 4). There was a drop in serum PTH by Day 16 of gestation (Fig. 5), but the decrease was not statistically significant.

Treatment of virgin rats with 100 mg/kg EGME did not affect serum concentrations of total calcium (Fig. 1), ionic calcium (Fig. 2), $25(OH)D_3$ (Fig. 3), $1,25(OH)_2D_3$ (Fig. 4), or PTH (Fig. 5). Likewise, there were no significant differences among the EGME treatment groups in these serum variables on gestation Day 16. On Day 21 of gestation, there appeared to be a dose-dependent effect of EGME treatment on most of the serum variables examined. Total calcium (Fig. 1) and ionic calcium (Fig. 2) were significantly increased, while $1,25(OH)_2D_3$ was significantly decreased (Fig. 4) in rats treated with 100 mg/kg EGME. $25(OH)D_3$ (Fig. 3) was increased, but not significantly. PTH tended to be lower in EGME-treated dams (Fig. 5), but the decrease was not statistically significant.

DISCUSSION

Halloran and DeLuca (1980b) demonstrated that vitamin D deficiency during pregnancy, which markedly reduced serum calcium concentration, had a deleterious effect on reproductive outcome. However, maternal morbidity and mortality were associated with the reduced litter size and pup weight. In the present study maternal toxicity was negligible, although EGME was clearly embryotoxic. The highest dose (100 mg/kg) caused all fetuses to be resorbed. The low dose (50 mg/kg) caused a small, but statistically significant, decrease in viable fetuses, and a significant increase in visceral malformations. We have recently reported a similar outcome following treatment with 50 mg/kg EGME

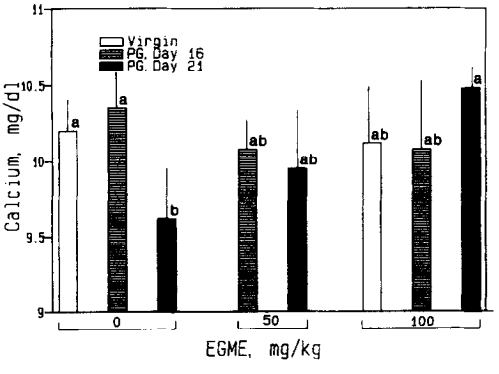

FIG. 1. Serum concentrations of total calcium in virgin rats and pregnant dams on gestation Days 16 and 21. Virgin rats were treated for 7 days with EGME, and pregnant dams were treated with EGME on gestation Days 9–15. Each bar represents the mean ± SD of six observations. Bars with a letter in common do not differ significantly ($p < 0.05$).

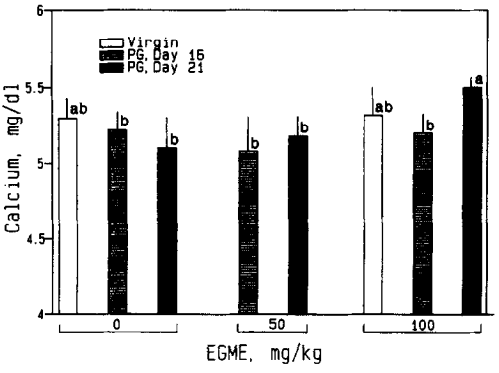

FIG. 2. Serum concentrations of ionic calcium in virgin rats and pregnant dams on gestation Days 16 and 21. Virgin rats were treated for 7 days with EGME, and pregnant dams were treated with EGME on gestation Days 9–15. Each bar represents the mean ± SD of six observations. Bars with a letter in common do not differ significantly ($p < 0.05$).

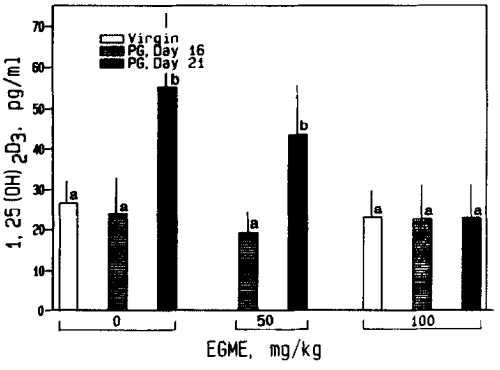

FIG. 4. Serum concentrations of $1,25(OH)_2D_3$ in virgin rats and pregnant dams on gestation days 16 and 21. Virgin rats were treated for 7 days with EGME, and pregnant dams were treated with EGME on gestation Days 9–15. Each bar represents the mean ± SD of six observations. Bars with a letter in common do not differ significantly ($p < 0.05$).

on gestation Days 7–13 (Toraason *et al.*, 1985). Treatment of virgin rats with 100 mg/kg EGME had no apparent effects, as indicated by normal body, kidney, and liver weights.

The reduced liver weight on gestation Day 21 in dams treated with 100 mg/kg EGME may be associated directly with loss of litters. Compared with that in virgin rats, liver weight in control dams and in dams treated with 50 mg/kg EGME increased over 25% by gestation Day 16 (data not shown), and increased at an additional 25% by gestation Day 21. In pregnant dams treated with 100 mg/kg EGME, liver weight increased 25% by gestation Day 16, but did not increase further during the last 4 days of pregnancy while fe-

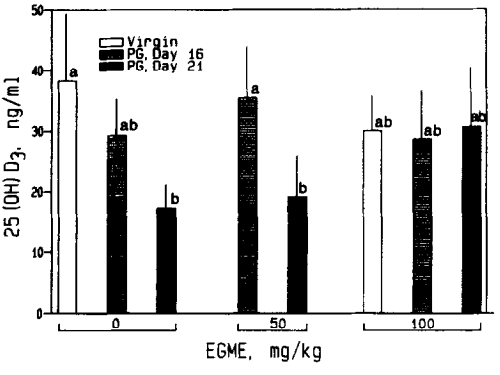

FIG. 3. Serum concentrations of $25(OH)D_3$ in virgin rats and pregnant dams on gestation Days 16 and 21. Virgin rats were treated for 7 days with EGME, and pregnant dams were treated with EGME on gestation Days 9–15. Each bar represents the mean ± SD of six observations. Bars with a letter in common do not differ significantly ($p < 0.05$).

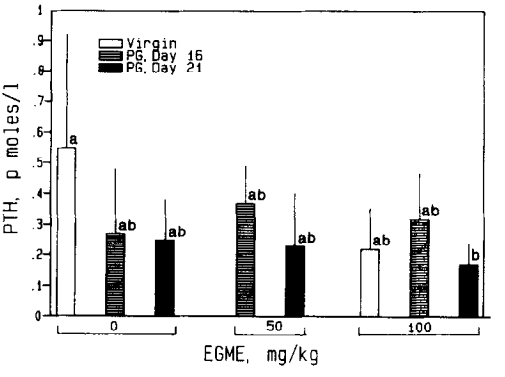

FIG. 5. Serum concentrations of PTH in virgin rats and pregnant dams on gestation Days 16 and 21. Virgin rats were treated for 7 days with EGME, and pregnant dams were treated with EGME on gestation Days 9–15. Each bar represents the mean ± SD of six observations. Bars with a letter in common do not differ significantly ($p < 0.05$).

tuses were being resorbed. Liver weight was reduced only in that group in which all litters were completely resorbed.

During the first 16 days of gestation dams appear to be capable of meeting the calcium requirements of the offspring with little or no adjustment in calcium homeostasis. Between Days 16 and 21, the rapid growth of fetuses requires activation of calcium homeostatic mechanisms. The changes in serum concentrations of calcium and vitamin D metabolites in near-term-pregnant rats in the present study are similar to those reported elsewhere (Halloran *et al.*, 1979). $1,25(OH)_2D_3$ increased significantly, and total calcium and $25(OH)D_3$ decreased significantly. PTH and ionic calcium were also decreased but not significantly.

The statistically significant adjustments in calcium homeostasis in the control rats are closely associated with the marked increase in gravid uterus weight between Days 16 and 21 of gestation. The weight increase was due to the rapid growth of fetuses, which put a high demand on maternal calcium stores. In rats treated with 100 mg/kg EGME, gravid uterus weight decreased during the last 4 days of gestation. The loss of litters reduced the demand for calcium and dams were able to maintain serum calcium levels at nonpregnant levels. Therefore, there was not a need for enhanced conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, serum concentration of $25(OH)D_3$ did not decline, and $1,25(OH)_2D_3$ did not increase. The absence of homeostatic adjustment at Day 21 in dams treated with 100 mg/kg EGME resulted in serum concentrations of calcium and vitamin D metabolites that were significantly different from those of pregnant rats treated with 0 mg/kg EGME.

The concentration of PTH in serum during pregnancy has been reported to rise above and fall below nonpregnant levels (Cushard *et al.*, 1972; Pitkin, 1975; Garel, 1983). In the present study, both pregnancy and EGME treatment appeared to reduce serum PTH. However, only on gestation Day 21 in rats receiving 100 mg/kg EGME was PTH significantly below that found in virgin control rats. Why PTH levels did not correspond with the readjustment in serum concentrations of calcium and vitamin D metabolites after loss of litters is not evident from the present data.

In summary, EGME is strongly embryotoxic in the absence of maternal toxicity. Complete loss of litters negates the high demand that late fetal development exerts on maternal calcium stores. As a result, dams do not exhibit the normal shift in calcium homeostasis generally associated with pregnancy. The absence of a shift in calcium homeostasis in this study appears to be secondary to the EGME-induced loss of litters.

REFERENCES

BOASS, A., TOVERUND, S. U., MCCAIN, T. A., PIKE, J. W., AND HAUSSLER, M. R. (1977). Elevated serum levels of 1,25-dihydroxycholecalciferol in lactating rats. *Nature (London)* **267,** 630–632.

CUSHARD, W. G., JR., CREDITOR, M. A., CANTEBURY, J. M., AND REISS, E. (1972). Physiologic hyperparathyroidism in pregnancy. *J. Clin. Endocrinol. Metab.* **34,** 767–771.

GARABEDIAN, M., HOLICK, M. F., DELUCA, H. F., AND BOYLE, I. T. (1971). Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. *Proc. Natl. Acad. Sci. USA* **69,** 1673–1679.

GAREL, J.-M. (1983). Parathyroid hormone, calcitonin and mineral metabolism in the mammalian fetus and neonate. In *Perinatal Calcium and Phosphorus Metabolism* (M. F. Holick, C. S. Anast, and T. K. Gray, eds.), pp. 78–104. Elsevier, New York.

HALLORAN, B. P., BARTHELL, E. N., AND DELUCA, H. F. (1979). Vitamin D metabolism during pregnancy and lactation in the rat. *Proc. Natl. Acad. Sci. USA* **17,** 5549–5553.

HALLORAN, B. P., AND DELUCA, H. F. (1980a). Calcium transport in small intestine during pregnancy and lactation. *Amer. J. Physiol.* **239,** E64–E68.

HALLORAN, B. P., AND DELUCA, H. F. (1980b). Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. *J. Nutr.* **110,** 1573–1580.

HARDIN, B. D. (1983). Reproductive toxicity of the glycol ethers. *Toxicology* **27,** 91–102.

HOBSON, D. W., D'ADDARIO, A. P., AND UDDIN, D. E. (1984). A comparative 90-day dermal exposure study of diethylene glycol monomethyl ether (DEGME) and

ethylene glycol monomethyl ether (EGME) in the guinea pig. *Toxicologist* **4,** 184 (abstract).

NAGANO, K., NAKAYAMA, E., OOBAYASHI, H., YAMADA, T., ADACHI, H., NISHIZAWA, T., OZAWA, H., NAKAICHI, M., OKUDA, H., MINAMI, K., AND YAMAZAKI, K. (1981). Embryotoxic effects of ethylene glycol monomethyl ether in mice. *Toxicology* **20,** 335–343.

NELSON, B. K., AND BRIGHTWELL, W. S. (1984). Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers. *Environ. Health Perspect.* **57,** 43–46.

NELSON, B. K., SETZER, J. V., BRIGHTWELL, W. S., MATHINOS, P. R., KUCZAK, M. H., WEAVER, T. E., AND GOAD, P. T. (1984). Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. *Environ. Health Perspect.* **57,** 261–271.

NIOSH (1983). The glycol ethers, with particular reference to 2-methoxyethanol and 2-ethoxyethanol: Evidence of adverse reproductive effects. DHHS (NIOSH) publication No. 83-112.

PIKE, J. W., PARKER, J. B., HAUSSLER, M. R., BOASS, A., AND TOVERUND, S. U. (1979). Dynamic changes in circulating 1,25-dihydroxyvitamin D during reproduction in rats. *Science* **204,** 1427–1429.

PITKIN, R. M. (1975). Calcium metabolism in pregnancy: A review. *Amer. J. Obstet. Gynecol.* **121,** 724–737.

TORAASON, M. (1983). Calcium flux *in vivo* in the rat duodenum and ileum during pregnancy and lactation. *Amer. J. Physiol.* **245,** G624–G627.

TORAASON, M., STRINGER, B., STOBER, P., AND HARDIN, B. D. (1985). Electrocardiographic study of fetuses exposed to ethylene glycol monomethyl ether (EGME). *Teratology* **32,** 33–39.

WILSON, J. G. (1965). Embryological considerations in teratology. In *Teratology—Principles and Techniques* (J. C. Wilson and J. Warkany, eds.), pp. 251–261. Univ. of Chicago Press, Chicago.