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# ORNITHINE DECARBOXYLASE ACTIVITY IN THE NEONATAL RAT HEART FOLLOWING PRENATAL EXPOSURE TO ETHYLENE GLYCOL MONOMETHYL ETHER

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## ABSTRACT

In mammals, ornithine decarboxylase (ODC) activity is highest during periods of rapid cellular growth and development, and the normal pattern of ODC activity during this period is sensitive to chemical and drug exposure. The industrial solvent ethylene glycol monomethyl ether (EGME) is teratogenic to rats and mice, with the heart being particularly sensitive. Basal ODC activity and ODC activity following an isoproterenol challenge were used to assess heart function in 3-, 9-, 16- and 22- day-old offspring from dams treated with 25 mg/kg EGME by gavage on days 7-13 or 13-19 of gestation. Reproductive outcome was not affected by EGME and none of the offspring had gross physical abnormalities. Gestation length was prolonged by both EGME treatments, but the increase was statistically significant only in the group treated on days 7-13 gestation. ODC activity per mg protein was greatest in 3-day-old rats and dropped off sharply during the following 3 weeks. In 3-day-old rats exposed on days 7-13 of gestation, ODC activity was 54% of that found in controls. ODC activity was comparable to that in controls in 3-day-old rats exposed on days 13-19 of gestation. Isoproterenol increased ODC activity in all groups, but additional functional abnormalities were not revealed by the isoproterenol challenge.



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## INTRODUCTION

Ornithine decarboxylase (ODC) has a number of characteristics that make it useful in developmental studies. ODC is essential in mammalian cells for the conversion of ornithine to putrescine[1], which is the rate limiting step in the metabolism of the polyamines spermadine and spermine[2,3]. ODC activity is highest in rapidly growing tissues such as regenerating liver, tumors and embryos[4,5,6]. Enhanced ODC activity can be induced by various drugs, hormones, cyclic nucleotides and calcium[1,7,8]. The importance of ODC to development has been made evident by the action of DL-α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC activity. Prenatal administration of DFMO arrests embryonic development in mice, rats and rabbits[9,10], while postnatal treatment with DFMO causes deficiencies in heart, brain and kidney growth in neonates[11].

A number of studies have demonstrated that the characteristic pattern of ODC activity in brain and heart during fetal and neonatal development is sensitive to prenatal exposure to drugs and alcohol[12,13,14,15]. ODC activity has also been used as an index of heart tissue response to β-adrenergic stimulation[16,17,18,19]. The importance of ODC activity to the developmental process, its sensitivity to prenatal exposures, and its responsiveness to physiological stimulation makes ODC a potentially useful marker for evaluating the effects of reproductive toxins on cellular function during development.

Ethylene glycol monomethyl ether (EGME), also known as 2-methoxyethanol, is used as an anti-icing additive in various fluids and fuels, and as a solvent in many lacquers, enamels, varnishes, inks and dyes[20]. EGME has repeatedly been shown to be a teratogen in rats and mice with the cardiovascular system being particularly sensitive[21,22,23]. We[24] have found that treatment of dams with 25 or 50 mg/kg EGME on days 7-13 of

gestation significantly increased the incidence of abnormal electrocardiograms (EKG's) in offspring. In addition to the aberrant EKG's, the 50 mg/kg treatment caused gross external malformations as well as visceral abnormalities (primarily cardiovascular). In contrast, the 25 mg/kg treatment produced aberrant EKG's in the absence of a significant increase in gross or visceral abnormalities. This led to the hypothesis that other functional deficiencies would be evident in the heart at an EGME exposure level that did not produce overt signs of toxicity. In the present study, basal (nonstimulated) heart ODC activity and ODC activity following an isoproterenol challenge were used to assess the effects of prenatal EGME exposure on heart function in neonatal rats.

## MATERIALS AND METHODS

Sprague Dawley rats were obtained from Charles River Breeding Labs, Wilmington, MA, and maintained on a 12 hr light--12 hr dark photoperiod at 23 $\pm$ 2$^{o}$ C and 55 $\pm$ 15% relative humidity. Rat chow (Ralston Purina Co., St. Louis, MO) and tap water were provided *ad libitum*. Two virgin females were cohabited with each male. The following day, sperm positive females were randomly divided into three groups (13 per group), and housed individually in wire mesh cages. The control group received 10 ml distilled water per kg body weight by gavage on days 7-19 of gestation (sperm = day 1). The exposure groups received 25 mg/kg EGME in 10 ml/kg water on days 7-13 or 13-19 of gestation. EGME was purchased from and certified by Fisher Scientific Company, Fair Lawn, New Jersey. On day 19 of gestation, females were moved to plastic cages with nonpyrogenic sawdust bedding.

Starting on day 20, pregnant dams were checked for delivery before noon and again at 6 PM. One half day was added to the

delivery time of females that did not finish delivering by noon. When all pups were delivered and cleaned by the mother they were counted and weighed. Delivery day was considered day 1. On day 2, all litters were randomly culled to 10 pups, which was the size of the smallest litter. Pups were sacrificed on days 3, 9, 16 or 22.

On the day of sacrifice [for heart ODC determination], 2 pups were chosen randomly from each litter. One pup was administered 10 mg/kg isoproterenol (Sigma, St. Louis, MO) in 10 ml/kg distilled water by i.p. injection, while its littermate received only water. Pups were killed 4 hrs later by decapitation. Hearts were removed, trimmed of atria, rinsed in ice cold 10 mM Tris buffer (Tham, Fisher Scientific), blotted on absorbent paper and weighed to the nearest mg. Hearts from 3-, 9-, 16- and 22-day-old pups were homogenized (Polytron) respectively in 2, 3, 4 and 5 ml of ice cold 10 mM Tris (pH 7.2). The homogenate was spun at 13,000 $x_g$ for 30 min. Two 0.5 ml aliquots of supernatant from each heart were frozen in 15 ml polystyrene centrifuge tubes at -70° C[25].

Three to 4 weeks later the supernatant was thawed at room temperature and assayed for ODC activity by generating $^{14}CO_2$ from DL(1-$^{14}$C)ornithine by a modification[14] of the method of Russell and Snyder[4]. One half ml of freshly prepared incubation medium was added to each 0.5 ml of supernatant. The incubation medium consisted of 0.1 mM Tris, 0.01 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM pyridoxal 5'-phosphate , 0.2 mM unlabeled ornithine and 0.5 μCi/ml DL(1-$^{14}$C)ornithine (specific activity, 55.7 mCi/mmole, Amersham, Arlington Heights, IL). The tubes were immediately capped with rubber stoppers with attached center wells (Kontes, Vineland, NJ) containing 0.2 ml hyamine hydroxide (Sigma, St. Louis, MO). Tubes were incubated for 30 min at 37° C in a shaking water bath. The reaction was stopped by injecting 1 ml of 2 M citric acid through the rubber stopper into

the incubation medium. Tubes were removed from the incubator and allowed to sit at room temperature for 60 min. Rubber stoppers were removed and the center wells containing hyamine hydroxide and trapped $^{14}CO_2$ were snipped from the caps and placed in scintillation vials containing 10 ml of Permafluor (Packard, Downers Grove, IL). Samples were counted on a Beckman scintillation spectrometer (Palo Alto, CA). ODC activity is presented as pmoles $CO_2$/30 min/mg protein. Protein content of the supernatant was determined by the method of Bradford[26].

Variables were examined for treatment effects using the Kruskal-Wallis test. Where effects were found ($p < 0.05$), each EGME treatment was compared to the control using Dunn's method of multiple comparisons. Because of the conservative nature of the Dunn's method[27], individual treatments were considered significantly different than control if $p < 0.1$.

## RESULTS

Exposure to EGME did not affect maternal body weight, litter weight or number of viable offspring (Table 1). EGME prolonged gestation in both treatment groups, but the increase was statistically significant only in the group treated on days 7-13 of gestation (Table 1). Body (Fig. 1) and heart (Fig. 2) weight of neonates during the first 22 days of life were not affected by the in utero exposures to EGME.

Basal heart ODC activity during the first 22 days is shown in Figure 3. ODC activity was greatest in 3-day-old rats, and dropped off markedly as rats aged. This pattern was evident in the controls as well as the two exposed groups. In rats exposed on days 13-19 of gestation, ODC activity was comparable to control values at the times examined. In rats exposed to EGME on gestation days 7-13 (Fig. 3B), ODC activity was significantly

TABLE 1
REPRODUCTIVE OUTCOME

| EGME Treatment | 0 mg/kg, Days 7-19 | 25 mg/kg, Days 7-13 | 25 mg/kg, Days 13-19 |
|---|---|---|---|
| Dams Exposed | 13 | 13 | 13 |
| Litters Delivered | 12 | 13 | 12 |
| Maternal Wt, g | | | |
| Gestation Day 13 | 244 ± 4 | 247 ± 5 | 256 ± 3 |
| Gestation Day 20 | 311 ± 5 | 316 ± 7 | 319 ± 4 |
| Post Delivery | 237 ± 4 | 238 ± 6 | 249 ± 5 |
| Litter Size | 12.2 ± 0.3 | 13.5 ± 0.5 | 12.8 ± 0.4 |
| Litter Wt, g | 75.1 ± 2.5 | 81.5 ± 2.4 | 79.8 ± 2.4 |
| Gestation Length, days | 22.0 ± 0.2 | *22.7 ± 0.3 | 22.3 ± 0.1 |

Values are mean ± SE. * Significantly greater than control. See text for statistical tests and level of significance used.

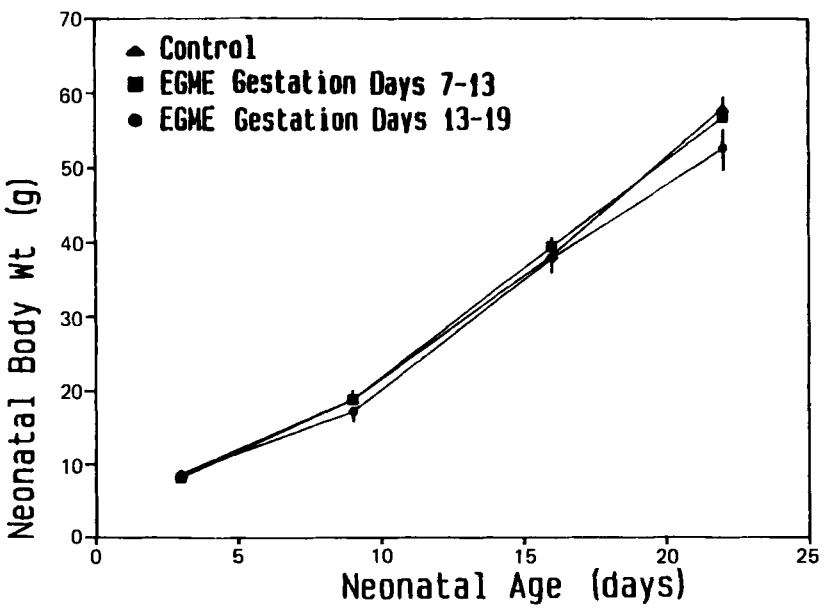


Figure 1. Body weights of neonatal rats from dams exposed to 25 mg/kg EGME on gestation days indicated. Values are mean ± SE, n = 12-13.

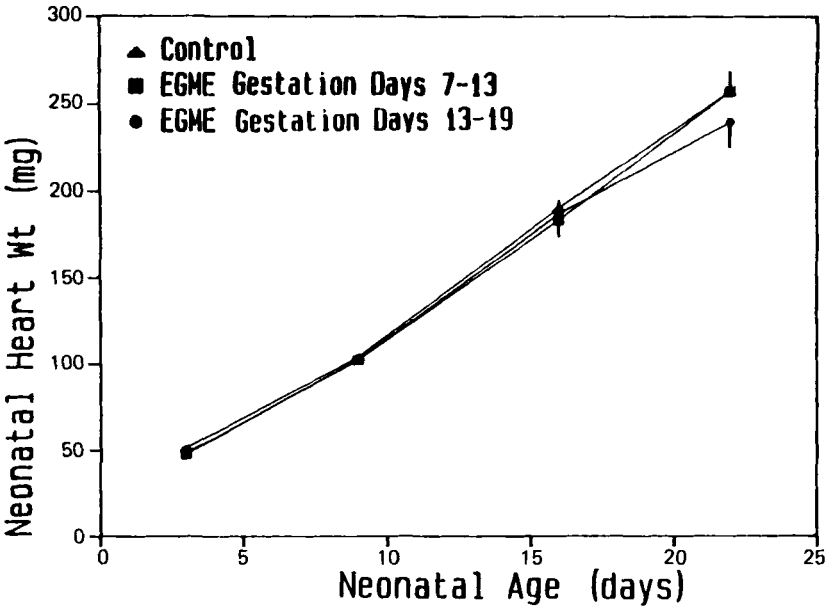


Figure 2. Heart weights of neonatal rats from dams exposed to 25 mg/kg on gestation days indicated. Values are mean ± SE, n = 12-13.

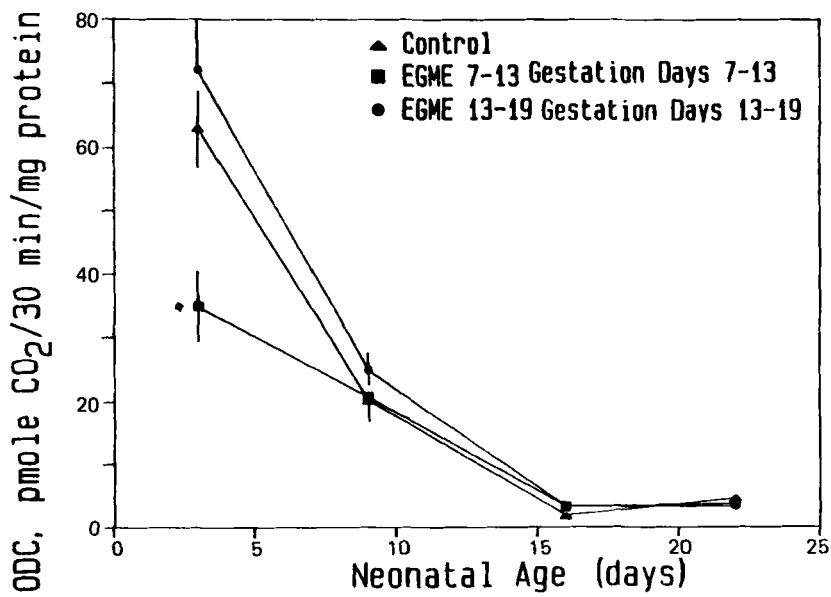


Figure 3. Basal ODC activity in neonatal rats from dams exposed to 25 mg/kg EGME at times shown. Values are mean ± SE, n = 12-13. *Significantly different from control. See text for statistical tests and level of significance used.

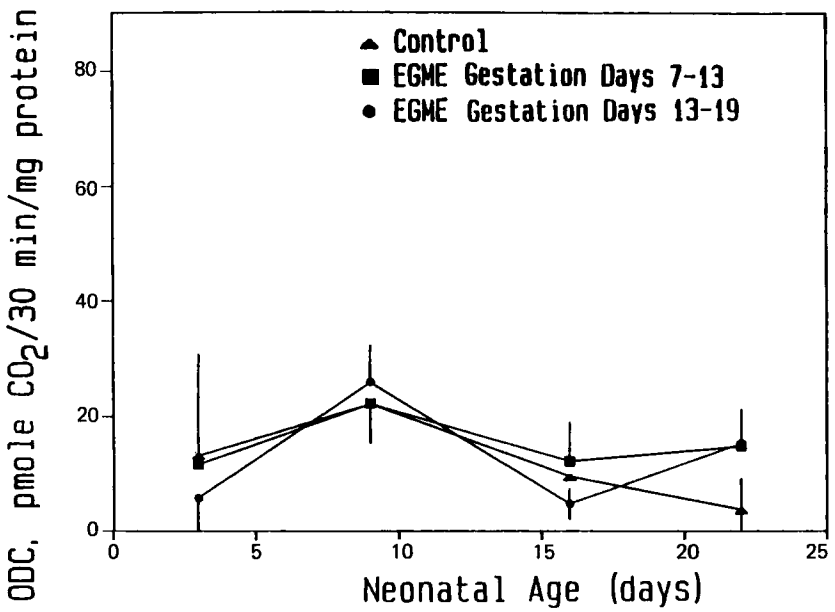


Figure 4. Net increase in heart ODC activity 4 hrs after administration of isoproterenol. Values are the difference between basal ODC activity shown in Fig. 3 and the ODC activity of littermates administered 10 mg/kg isoproterenol by I.P. injection (data not shown). Values are mean $\pm$ SE, n = 12-13.

below the control value when rats were 3 days old. This was the case for both the water treated (basal activity) and isoproterenol treated neonates (data not shown). At all other times and treatments, ODC activity was comparable to ODC activity in controls.

Isoproterenol increased ODC activity in all groups at all time periods examined. Figure 4 illustrates the mean net response to isoproterenol administration. The response to isoproterenol remained relatively constant during the first 21 days of life. Exposure to EGME either early or late in gestation did not significantly affect the the heart's ODC response to isoproterenol.

## DISCUSSION

In a recent behavioral teratology study, Nelson and Brightwell[28] exposed dams to 25 ppm EGME on days 7-13 or 14-20 of gestation. The only effects found were behavioral modifications and altered levels of neurotransmitters in brain tissue of the offspring. Nelson and Brightwell[28] noted that their findings support the proposal that functional assessment is a more sensitive indicator of prenatal insult than conventional indices of developmental toxicity. Similarly, 25 mg/kg EGME administered by gavage in the present study did not affect maternal body weight or reproductive outcome. None of the exposed pups had gross physical defects, and heart and body growth were normal during the first 22 days of life. These findings indicate that these treatments were not overtly toxic to the dams or their offspring. However, EGME exposure did prolong gestation in the dams and did alter the normal pattern of ODC in the neonatal heart.

EGME[22] and the structurally related glycol ether, ethylene glycol monoethyl ether (EGEE)[29,30,31] have been shown to increase gestation length. Hardin[22] suggested that the prolonged gestation following exposure to the glycol ethers could be due to impaired uterine smooth muscle contractility, since several glycol ethers have been found to depress smooth muscle activity[32]. However, if prolonged gestation in the present study was due solely to inhibition of uterine contractility then the late gestation exposure to EGME would have been more effective. Why the earlier exposure was more effective is not clear, but it may stem from an effect of EGME on the fetuses.

Offspring from mothers treated on days 7-13 of gestation with EGME had normal body weights despite being born late. This suggests that toward the end of gestation, fetuses exposed early in gestation to EGME were undersized. This is consistent with

previous observations where EGME exposure early in gestation reduced body weight in 20-day-old fetuses (Toraason, unpublished). To what extent the prolonged gestation was due to a possible retarded growth of the fetus is not apparent.

ODC activity begins to increase during organogenesis, peaks during embryogenesis and then sharply declines[4,5,17]. Heart ODC activity measured in the present study illustrates the end of this decline. The decreased heart ODC activity found in 3-day-old rats does not appear to be the result of direct action of EGME on the enzyme since ODC activity was unaffected in rats treated on days 13-19 of gestation. It is possible that the early exposure indirectly inhibited the normal increase during organogenesis. As a result, maximum ODC activity may not have been achieved during embryogenesis and the effect was still evident 3 days after birth. The prolonged gestation and the decreased heart ODC activity were apparently not directly related since gestation length and ODC activity were not correlated as determined by least squares regression ($r = 0.06$).

Buelke-Sam et al.[15] reported that prenatal reserpine exposure reduced heart ODC activity in neonatal rats. As in the present study, they found that ODC activity was reduced in 1-day-old neonates exposed to reserpine when control ODC activity was high, but there was no difference between controls and exposed rats when rats were 20 days old and ODC activity had dropped off. They suggested that challenging the heart to determine if normal functional responses were intact would provide more information on the postnatal toxicity of a prenatal exposure.

Catecholamines not only affect the chronotropic and inotropic action of the heart, they also stimulate ODC activity[19], and a tissue response can be demonstrated as early as day 13 of gestation in the fetal mouse[18]. As a result, stimulation of fetal ODC activity has been used as an index of cardiac

responsiveness to adrenergic stimulation during development[17]. In the present study, an isoproterenol challenge was used to stimulate ODC activity above basal levels. Despite the significant reduction in ODC activity in 3-day-old rats exposed to EGME on days 7-13 of gestation, the stimulation of ODC by an isoproterenol challenge was not impaired. The isoproterenol response in 3-day-old rats was also similar to controls. This indicates that the heart's autonomic nervous system response was not affected by the EGME exposure. It also suggests that the decreased ODC activity in the 3-day-old rats is only a developmental alteration rather than permanent functional impairment.

In summary, an EGME exposure that is not overtly toxic or teratogenic can disrupt the normal pattern of heart ODC activity. The stimulation of ODC by isoproterenol did not unmask any additional functional effects, and the reduced ODC activity did not appear to be associated with the prolonged gestation. These effects of EGME exposure are apparently of little consequence since viability, body weight and heart weight were normal during the first 22 days of life. If ODC activity is to be used in risk assessment it will be necessary to use dose levels that provide a range of toxicity to determine if alterations in ODC activity occur in the presence and absence of more overt toxicity.

## REFERENCES

1. Pegg, A.E. and McCann, P.P. (1982) Polyamine metabolism and function. Am. J. Physiol. 243, C212-C221.

2. Tabor, H. and Tabor, C.W. (1964). Spermidine and spermine and related amines. Pharmacol. Rev. 16, 245-300.

3. Raina, A. and Janne, J.. (1970) Polyamines and the accumulation of RNA in mammalian systems. Fed. Proc. 29, 1568-1574.

4. Russell, D. and Snyder, S.H. (1968). Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. Proc. Natl. Acad. Sci. USA. 60, 1420-1427.

5. Snyder, S.H. and Russell, D.H. (1970). Polyamine synthesis in rapidly growing tissues. Fed. Proc. 29, 1575-1582.

6. Russell, D.H. and Durie, B.G.M. (1978). Polyamines as biochemical markers of normal and malignant growth. In: Progress in Cancer Research, Vol. 8. Raven Press, New York, pp. 1-178.

7. Russel, D.H. (1980). Ornithine decarboxylase as a biological and pharmacological tool. Pharmacology 20, 117-129.

8. Langdon. R.C., Fleckman, P. and McGuire, J. (1984). Calcium stimulates ornithine decarboxylase activity in cultured mammalian epithelial cells. J. Cell. Physiol. 118, 39-44.

9. Fozard, J.R., Part, M.-L., Prakash, N.J., Grove, J., Schechter, P.J., Sioerdsma, A. and Koch-Weser, J. (1980). L-ornithine decarboxylase: and essential role in early mammalian embryogenesis. Science 208, 505-508.

10. Fozard, J.R., Part, M.-L., Prakash, N.J., Grove, J. (1980). Inhibition of murine embryonic development by a-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase. European J. Pharmacol. 65, 379-391.

11. Slotkin, T.A., Persons, D., Slepetus, R.J., Taylor, D. and Bartolome, J. (1984). Control of nucleic acid and protein synthesis in developing brain, kidney, and heart of the neonatal rat: effects of a-difluoromethylornithine, specific, irreversible inhibitor of ornithine decarboxylase. Teratology 30, 211-224.

12. Thadani, P.V., Lau, C., Slotkin, T.A. and Schanburg, S.M. (1977). Effect of maternal ethanol ingestion on neonatal rat brain and heart ornithine decarboxylase. Biochem. Pharmacol. 26, 523-527.

13. Slotkin, T.A., Seidler, F.J. and Whitmore, W.L. (1980). Effects of maternal methadone administration on ornithine decarboxylase in brain and heart of the offspring: relationships of enzyme activity to dose and to growth impairment in the rat. Life Sci. 26, 861-867.

14. Thadani, P.V. (1983). Prenatal ethanol exposure alters development of heart ornithine decarboxylase response to insulin in rat.II. Daily dose. Res. Comm. Chem. Pathol. Pharmacol. 41, 19-36.

15. Buelke-Sam, J., Kimmel, G.L., Webb, P.J., Slikker, Jr., W., Newport, G.D., Nelson, C.J. and Kimmel, C.A. (1984). Postnatal toxicity following prenatal reserpine exposure in rats: effects of dose and dosing schedule. Fund. Appl. Toxicol. 4, 983-991.

16. Thadani, P.V. and Schanberg, S.M. (1979). Effect of stress and sympathetic activity on rat cardiac and aortic ornithine decarboxylase activity. Life Sci. 25, 1009-1016.

17. Haddox, M.K., Womble, J.R., Larson, D.F., Roeske, W.R. and Russell, D.H. (1981). Isoproterenol stimulation of ornithine decarboxylase blocked by propanolol during ontogeny of the murine heart. Mol. Pharmacol. 20, 382-386.

18. Copeland, J.G., Larson, D.F., Roeske, W.R., Russell, D.H. and Womble, J.R. (1982). $\underline{B_2}$-adrenoceptors regulate induction of myocardial ornithine decarboxylase in vivo. Br. J. Pharmac. 75, 479-483.

19. Lau, C. and Slotkin, T.A. (1982). Stimulation of rat heart ornithine decarboxylase by isoproterenol: evidence for post-translational control of enzyme activity? European J. Pharmacol. 78, 99-105.

20. 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.

21. 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.

22. Hardin, B.D. (1983). Reproductive toxicity of the glycol ethers. Toxicology 27, 91-102.

23. 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.

24. 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.

25. Gaines, D. and Friedman, L. (1984). Effect of storage and freezing and thawing on ornithine decarboxylase (ODC) activity in rat tissue preparations. Fed. Proc. 43, 371 (abstract).

26. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

27. Gibbons, J. (1976). Nonparametric methods for quantitative analysis. Holt, Rinehart and Winston, pp 114-118.

28. Nelson, B.K. and Brightwell, W.S. (1984). Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers. Environ. Health Perspect. 57, 43-46.

29. Nelson, B.K., Brightwell, W.S., Setzer, J.V., Taylor, B.J. and Hornung, R.W. (1981). Ethoxyethanol behavioral teratology in rats. Neurotoxicology 2, 231-249.

30. Nelson, B.K., Brightwell, W.S. and Setzer, J.V. (1982). Prenatal interactions between ethanol and the industrial solvent 2-ethoxyethanol in rats: Maternal and behavioral teratogenic effects. Neurobehav. Toxicol. Teratol. 4, 387-394.

31. Nelson, B.K., Brightwell, W.S., Setzer, J.V. and O'Donohue, T.L. (1984). Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol. Environ. Health Perspect. 57, 255-259.

32. von Oettingen, W.F. and Jirouch. E.A. (1931). The pharmacology of ethylene glycol and some of its derivatives in relation to their chemical constitution and physical chemical properties. J. Pharmacol. Exp. Ther. 42, 355-372.