Lithium Ion in Cleft Palate Teratogenesis in CD_1 Mice[1] (37653)

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In recent years, lithium has been found effective in controlling the manic depressive psychosis in man (1). Lithium is absorbed promptly from the gastrointestinal tract and is largely excreted by the kidney (2). As this drug may be given to women during the childbearing age, an evaluation of its possible teratogenicity is important. Reports on such actions of lithium salts in man and other mammals are, to date, conflicting.

In the human, teratogenic effects have been reported in 3 out of 60 patients who received lithium during pregnancy (3). Another case of a congenitally malformed child was reported by Vacaflor *et al.* (4) while Weinstein and Goldfield (5), Silverman *et al.* (6), and Nassr (7) reported on four normal children where the mothers had received lithium before and during pregnancy.

Trautner *et al.* (8) treated Wistar rats before, during and after gestation with lithium chloride in drinking water and found no malformations in the litters. However, while the newborns were of the same size and weight as untreated controls, there appeared to be a 15% reduction in the number of corpora lutea found at laparotomy. Johansen and Ulrich (9) found that lithium crossed the placental barrier in Wistar rats but no teratogenic effect was demonstrable in the offspring with doses of 1 to 2 meq/kg/day of lithium given throughout gestation. On the other hand, Wright *et al.* (10) injected LiCl interperitoneally in pregnant Sprague–Dawley rats and induced eye and auricular defects and cleft palate in the offspring. When the lithium salt was injected subcutaneously (11) in Wistar rats, only 1 out of 152 fetuses was abnormal.

In mice, injection of 1% of lithium carbonate into pregnant NMRI/Han white mice raised the number of resorptions but only 1 out of 79 fetuses was malformed (12). Bass *et al.* (13) also reported no teratogenic effects but did find a reduction in the number of living offspring of albino farm white mice which were given 2–4 mg of LiCl in their diets from the second to the seventh days of gestation. However, Szabo (14) was able to produce cleft palate in the offspring of lithium-intubated pregnant HaM/ICR mice.

The purpose of the present paper is to further evaluate the teratogenic effect of lithium salts in mice when the compound is injected subcutaneously.

Methods and Materials. The mice used were of the CD_1 strain (Charles River). All animals were fed water and Purina Chow *ad libitum*. The females were placed with males overnight and examined each morning for the presence of a copulation plug. When found, the female was isolated and this day was counted as day 0. The mice were divided into five groups. Three groups were injected with 15.5 mg of an aqueous solution of LiCl (Merck Chemical Company) on days 11 and 12, 12 and 13, or 11, 12, and 13 of gestation. One group of control animals were injected subcutaneously with an equal volume of the vehicle (sterile water U.S.P.) on days 11, 12, and 13 of gestation, and another group of control mothers was uninjected. The mothers were sacrificed one day before delivery. The fetuses were removed from the uteri, weighed, and examined for cleft palate and other gross malformations. The sex of the fetuses was determined by laparotomy. Randomly selected litters were processed from the control groups and

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TABLE I. Effect of Lithium Chloride (15.5 mg daily dose) on the Occurrence of Induced Cleft Palate in CD_1 Mice.

Substance injected	Days on which given	Number of mothers	Offspring				Resorptions	% of cleft palate
			Males		Females			
			Normal	Cleft palate	Normal	Cleft palate		
LiCl	11, 12	10	43	2	41	1	21	3.4%
LiCl	12, 13	10	49	2	53	6	11	7.2%
LiCl	11–13	10	44	9	46	7	12	15.1%
Vehicle	11–13	8	52	—	45	—	4	—
—	—	9	51	—	46	1	4	1%

those litters containing animals with cleft palate were processed by the alizarin red technique in order to determine whether any bone anomalies other than cleft palate were present.

Results and Discussion. The incidence of cleft palate in the offspring of lithium injected mice of the CD_1 strain is shown in Table I. The CD_1 strain was selected because of its very low incidence of spontaneous cleft palate. The dosage of lithium used is similar to that reported to be teratogenic to HaM/ICR mice (14). After the drug was administered for 2 days, on days 11 and 12 of gestation, the incidence of induced cleft palate was 3.4%. If the mothers were injected on days 12 and 13 the incidence of induced cleft palate was 7.2% and if the injection was given for three days, on days 11, 12, and 13, the incidence was 15.1%. Mothers injected with vehicle alone showed no offspring with cleft palate while one offspring with cleft palate was found in the noninjected groups. The sex of both affected and nonaffected offspring was examined. While there was an increase in resorption sites in all injected groups, the sex ratio remained close to the 50–50 ratio indicating that probably there was no selective resorption of either sex. The incidence of induced cleft palate was also distributed equally in both sexes. Cleft palate animals were present in the several litters as follows: 3 litters had affected offspring in the 11, 12 day group, 4 litters had affected offspring in the 12, 13 day group and 6 litters had affected offspring in the 11, 12, 13 day group. No additional abnormalities were found in the specimens cleared and examined with alizarin red staining.

Lithium has been reported to interfere with gestation and fetal development in some lower mammals (8, 10, 13, 14). Other investigators were unable to confirm these findings (9, 11, 12). In those cases in which teratogenesis was induced in mice, the drug was given for a long period of time during gestation. In the present experiment, the drug was given for the short period of time when CD_1 animals were found to be susceptible to drug-induced cleft palate formation (15). By reducing the time of exposure to the agent, the toxic effects on the mothers and fetuses could be kept lower than those reported by Szabo (14). The dose used was compatible with full health of the mothers and no mother died during gestation in any of the groups.

It is well known that different strains of mice have a different susceptibility to the same teratogenic agent (16). It is therefore possible that the CD_1 strain may show some susceptibility to the teratogenic action of lithium salts while other strains do not. This difference may explain some of the conflicting evidence on the teratogenic properties of lithium.

Summary. The teratogenic effects of lithium on CD_1 mice were studied. A dose of 15.5 mg of lithium chloride monohydrate in sterile water (Injection U.S.P.) was given each day for 2 or 3 days on days 11 through 13 of pregnancy. The mice were sacrificed on day 17 of pregnancy. The uteri were ex-

amined for resorption sites and the fetuses for malformations. The incidence of cleft palate in the offspring of mice injected on days 11, 12, and 13 was 15.1%; on days 12 and 13, 7.2%; and on days 11 and 12, 3.4%. Since the incidence of spontaneous cleft palate is very low in these mice, a teratogenic effect of lithium salts has been demonstrated. There was also an increase in resorption sites. The pregnant mothers did not show any evident ill effects from the lithium salt at the dosage given.

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