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TERATOGENIC EFFECTS OF LITHIUM CARBONATE ON DEVELOPMENT OF MOUSE EMBRYOS

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Abstract

Lithium is widely used as an effective drug in the treatment of manic depression in human beings, despite its teratogenic effects on the development of different vertebrate and human embryos. Teratogenic effects of lithium carbonate have been investigated on the development of Balb/C mouse embryos in this work. In all experiments, lithium carbonate dissolved in physiological saline solution and IP (Intraperitoneal) single dose injections were applied. In the first series of experiments, LD50 standard was determined to be 443 mg/kg of body weight. In the main series of experiments, a therapeutic dose of 300 mg/kg B. W., lithium carbonate was injected into mice from three groups on days 7.5, 8.5 and 9.5 of gestation, each group consisting of four pregnant females. Simultaneously, three separate control groups were used each animal of which received only physiological saline solution. Vaginal plug observation day was considered as day 0 of gestation. On day 15.5 of pregnancy, the embryos were extruded and morphological and histological studies were carried out on them. Each series of the experiments was repeated three times. Experimental embryos of day 7.5 of gestation showed bilateral and unilateral anophthalmia, microphthalmia, coelosomy, cleft lip and palate, day 8.5, exencephaly and day 9.5, 50% atrophied embryos. In addition to these findings, the embryos of days 7.5 and 8.5 showed a significant decrease in CR measurements and body weight. The results from determination of serum concentration of lithium showed that after 3-6 hours of injection on days 7.5 and 8.5, the element passed through the placenta and affected the anterior neural folds (day 7.5) and optic vesicles (day 8.5) then caused exencephaly and anophthalmia consecutively.

Introduction

For years, lithium has been known as an effective agent in the maldevelopment of lower animals as well as in mammals and other vertebrates. Treatment of isolated animal pole blastomeres of sea urchin embryo by lithium chloride has produced stomach and spicules from these cells [26], and, during gastrulation, extra embryonic gut [9]. This element has the same effect

Keywords: Teratogenesis; Lithium carbonate; Mouse embryos

on cell fate and pattern formation during mesodermal induction in amphibian embryos [7].

Lithium carbonate is widely used as a maintenance drug in psychiatry [15]; and passes through the placenta [16, 19, 22]. Szabo [20] observed cleft palate using 300-465 mg/kg body weight lithium carbonate in Ham/ICR mice on day 6-15 of gestation. Loevy [10] applied 15.5 mg lithium carbonate to the same mouse strain and also found cleft palate on day 11-15 of pregnancy. In 1982, Smithberg and Dixit [19] reported exencephaly and skeletal malformations using 5 mg for each animal in 129 mouse strain; this dose was an amount of half LD50 standard. Jurand [6] found that exencephaly, cranioschisis, rachischisis, kinking of the spinal cord and dilation of the fourth brain ventricle is brought about by IP application of 330-340 mg/kg lithium carbonate in JBT/Jd mouse strain at the very beginning of day 9 and four days later. Hansen *et al.*, [3] using an *in vitro* embryo culture system in both mice and rats in rat serum containing lithium carbonate on days 8-12 of gestation for 24 hours observed an unclosed neural tube, and postulated that rat embryos were more sensitive than those of the mice. Oral application of lithium carbonate in rats [22, 5] and rat, rabbit and monkey [2] did not show any toxic effects and they gave birth to healthy newborns. In addition to these findings, Marathe and Thomas [11], after oral application of 100 mg/kg lithium carbonate on days 5-10 of gestation in rats, reported a decrease in the implantation rate, fetal body weight and number of live infants, and an increase in skeletal mal- formations. Sharma and Rawat [18], by oral administration of 7 mg/kg in rats on days zero to nine, have reported an increase in the number of atrophied embryos, cleft palate, hepatomegaly, body weight decrease and defects in fingers and brain. Wright [25] observed teratogenic effects of lithium in Sprague Dawley rats as atrophied embryos, cleft palate and defects in external ears, while Johansen [4] did not see any defects in Wistar rats. All these reports reveal that rats are more sensitive than mice to the toxicity of lithium carbonate.

Materials and Methods

Balb/C mice strain at the age of 70-90 days were used in all experiments. Monogamous copulations were set up and the day of vaginal plug observation was determined as day zero of gestation. After determination of LD50 standard, an IP single-dose injection of 300 mg/kg body weight lithium carbonate dissolved in 0.1% physiological saline solution was injected into all experimental animals. The controls received only saline solution.

The experimental groups received lithium carbonate on days 7.5, 8.5 and 9.5 of gestation. The embryos were dissected from pregnant mothers on day 15.5 of gestation and were washed in saline solution. Crown-rump (C. R.) measurements by means of a calliper and body weight using analytical balance for each embryo were determined. After morphological observations and photography of the embryos under a Zeiss stereomicroscope, model DRC, they were fixed in Bouin's fluid. Dehydrations were carried out using different grades of ethanol, and paraffin wax blocks, cut at 6 μm thickness, and the H & E staining method were used in all experiments. Photomicrographs were taken under a Zeiss photomicroscope, model M3. Concentration rate of lithium in blood serum was determined 6 and 24 hours after injection. For this purpose, blood was drawn from the hearts of both experimental and control animals, centrifuged at 4000 rpm and compared using atomic absorption apparatus. Data analysis of this research work for statistical studies was carried out using a personal computer.

Results

LD50 Standard Determination

For determination of LD50 standard, the double integration method was used in this work. Seven groups, each including seven female mice at the age of 70-90 days, were selected and the animals of each group received the dose levels shown in Table 1. The control animals of each group received physiological saline. Initially, the amount of lithium used was 300 mg/kg of body weight. The LD50 standard was determined at 443 mg/kg of body weight.

Determination of Serum Lithium Concentration

According to Table 2, it is postulated that lithium carbonate after IP administration is absorbed by visceral peritoneum. Our results agree with the findings of Linden [8] who had estimated half life of lithium (7-24 hours) in the patients. According to our results, serum lithium in mice is in the range of therapeutic concentration in humans (Table 2).

Results on Day 7.5 of Gestation

Results of the studies of the embryos treated on day 7.5 of gestation show an 18% unilateral and bilateral anophthalmi (Figs. 1, 2, 8, 9). Histological observation did not show any eye primordia, but the other organs had developed normally. In four cases, the embryos had unilateral anophthalmia appearance and a small ectopic eye was hidden amongst the head mesenchyme (Fig. 10). The retina of such embryos

Table 1. Results of LD50 standard determination

Working dose	Dose level	Animals		No. of dead exp. mice	Probability	$\frac{1}{2}(P_i+P_i+1)=E$
		Exp.	Cont.			
0	360	6	6	0	0	0.3335
1	383	6	6	4	0.667	0.5
2	408	6	6	2	0.333	0.4165
3	434.8	6	6	3	0.5	0.5835
4	463.8	6	6	4	0.667	0.8335
5	493.2	6	6	6	1.00	
6	525.3	6	6	6	1.00	
						ΣE=2.667

Using the following formula:

Log(LD50) = LogD + working LD50 (d)

Where:

D = minimum administered dose

d = Log of constant index

working LD50 = final working LD50 - ΣE

Table 2. Results of determining serum lithium concentration

Serum concentration of lithium		Observations
6 hours after injection	24 hours after injection	
Undetermined	0.006-0.014	Controls
1.19-1.98	0.15-0.24	Experimentals single dose of 300 mg/kg

was quite disorganized and their sensory and pigmented cell layers were not detectable (Fig. 11). In two embryos of this case, fibrillation of the eye lens had not occurred and the cells showed spheroid shapes and vesiculations (Fig. 12). In all of the unilateral anophthalmia embryos (11 cases) the other eye seemed quite normal. In embryos of this series of experiments, two coelosomy (Fig. 3), cleft lip and palate (Fig. 4) and exencephaly (Fig. 5) individuals were observed (Table 3). Statistical analysis of C. R., body weight and placenta weight showed significant decrease in these experiments (Histograms, 1, 2 and 3).

Results on Day 8.5 of Gestation

In Table 4, the total number of normal and

Table 3. Number and percent of normal and defected embryos on day 7.5 of gestation

Observations	No.	Percent
Normal	77	72.6
Atrophied	6	5.7
Bilateral anophthalmia	8	7.5
Unilateral anophthalmia	11	10.4
Coelosomy	1	1.9
Exencephaly	1	0.95
Cleft Palate	1	0.95
Total	106	100

Table 4. Number and percent of normal and defected embryos on day 8.5 of gestation

Observations	Number	Percent
Normal embryos	43	56
Atrophied embryos	26	34
Exencephaly	6	8
Coelosomy	1	1.3
Total	76	99.3

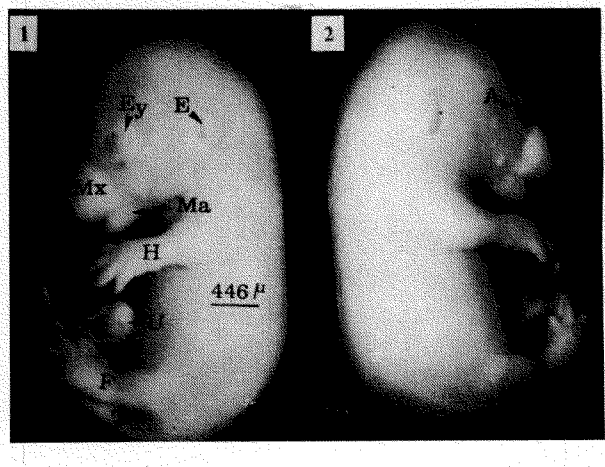


Figure 1. Serum injected control embryo on day 7.5 of gestation

Figure 2. Lithium injected embryo shows anophthalemia. A, position of anophthalmic eye.

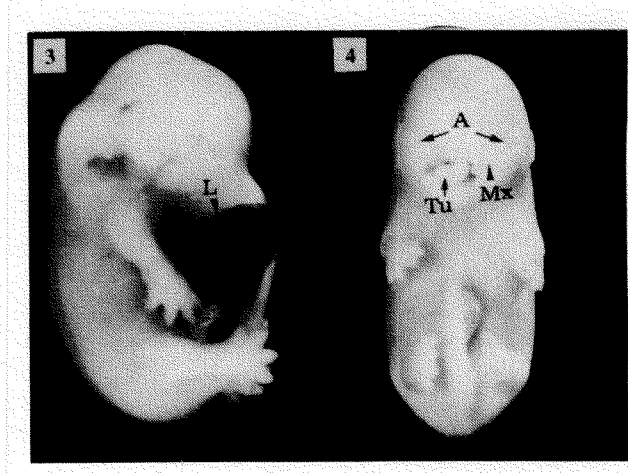


Figure 3. Lithium injected embryo with exohepaty (liver hernia). L, Liver

Figure 4. Lithium injected embryo with cleft lip and palate. Mx, maxilla; Tu, tongue

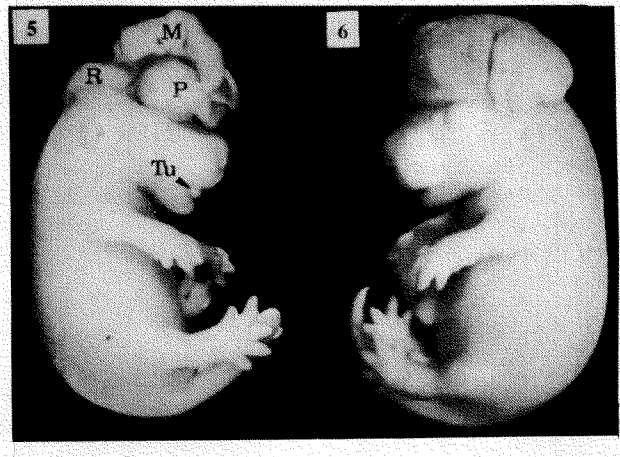


Figure 5. Lithium injected embryo with exencephaly (treated day 7.5)

Figure 6. Lithium injected embryo with exencephaly (treated day 8.5).

Figures 1-6. Stereophotomicrographs of control and experimental embryos, which have been treated on days 7.5, 8.5 and dissected on day 15.5 of gestation. X22.4 Ey, eye; E, ear; Mx, maxilla; Ma, mandible; Tu, tongue; H, upper limb; F, lower limb; U, umbilical herniatron; T, tail; L, liver; R, rhombencephalon (exencephalic); P, prosencephalon (exencephalic)

defected embryos is shown. There were 34% atrophied embryos in utero which was a considerable increase comparing with day 7.5. The main defect on day 8.5 was exencephaly (Fig. 6). Histological observations showed the brain defects (exencephalic embryos) from mesencephalon to rhombencephalon. The brain tissues had been formed out of cranium (Figs. 13a and 14a). Brain ventricles were smaller than normal. The bones of visceral cranium showed abnormal appearance and the angle between the atlas vertebra and viscerocranium was wider than in the control foetuses. Exencephalic brains did not show meninges on the outer surfaces (Figs. 13b and 14b) but the basal part of the brains near the viscerocranium showed a considerable increase in arachnoid layer (Fig. 14a). Besides the exencephaly defect, one case of coelosomy was observed in which part of the liver showed exohepaty. Statistical analysis on these series of experiments showed a significant decrease in C. R. measurements and body and placenta weight

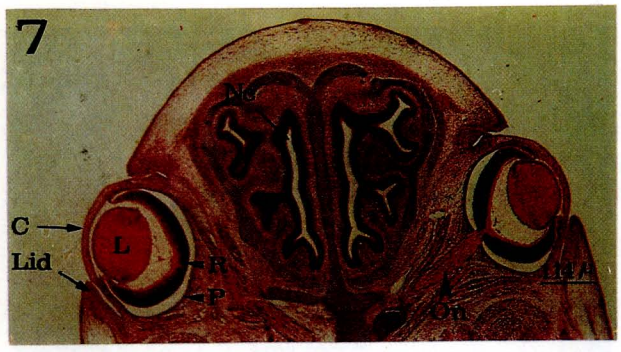


Figure 7. Control embryo (day 15.5)

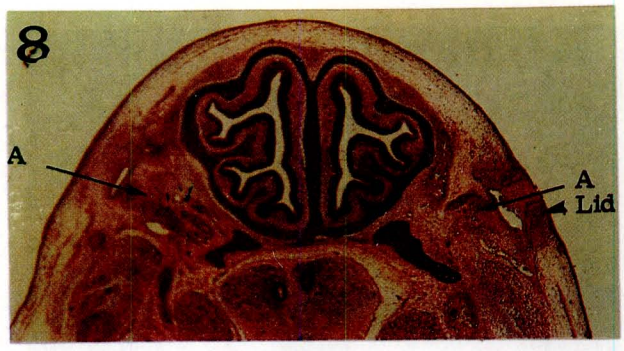


Figure 8. Experimental embryo with bilateral anophthalemia treated with lithium on day 7.5. A, anophthalmia; Lid, abnormal closure of the eyelid

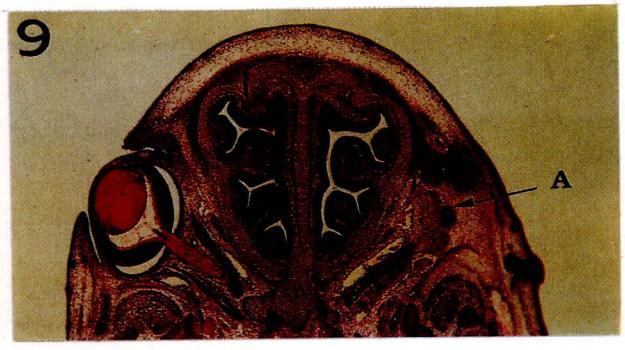


Figure 9. Experimental embryo with unilateral anophthalmia (A) treated with lithium on day 7.5 of gestation

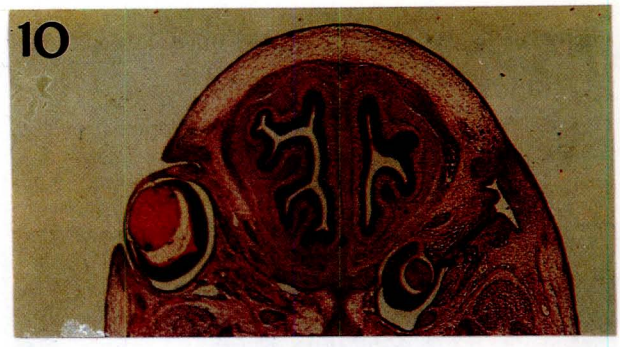


Figure 10. Experimental embryo with unilateral microphthalmia in left eye. (E). R, retina; A, undeveloped eye region; C, cornea, L, lens; NC, nasal cavity; P, pigmented layer; On, optic nerve; E, microphthalmic eye; Lid, eyelid.

Figures 7-10. Stereophotomicrograph of cross section from the eye region. X87.5

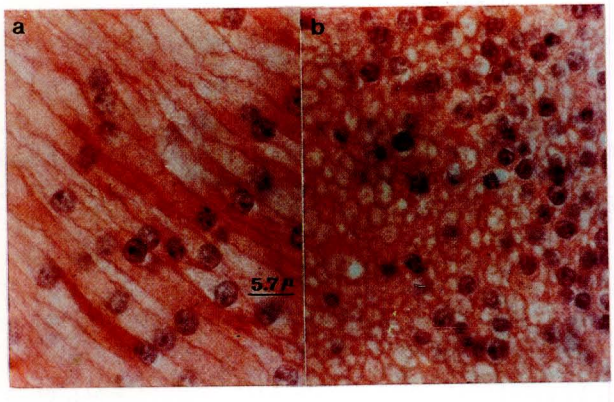


Figure 11. a) Photomicrograph of cross section of retina in control embryos (day 15.5 of gestation). b) Experimental embryo, cross section of the retina from the unilateral microphthalemic embryo (treated on day 7.5). Note the retinal cell disorganization. X1750

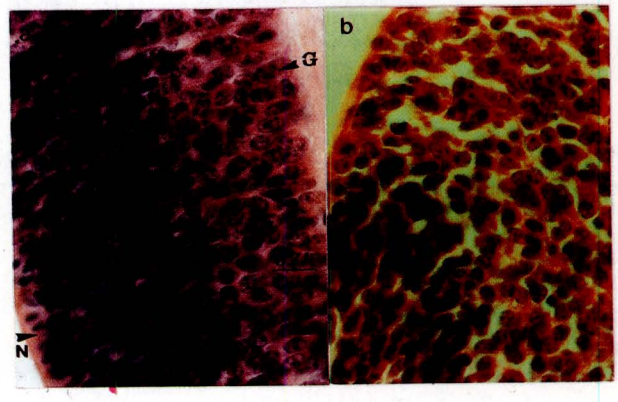


Figure 12. a) Photomicrograph of lens cross section in control embryo (day 15.5). b) Experimental embryo, cross section of the lens of a microphthalmic embryo treated on day 7.5. The fibrillar appearance of the lens fibres in control and spheroid shape of lens fibres in experimental are comparable. N, granular cell layer; G, ganglionic cell layer X1750

(Histograms 4, 5 and 6).

Results of Day 9.5 of Gestation

Table 5 shows the number of normal and defected embryos on day 9.5. In these series of experiments, considerable defects in the embryos were not noted, but 50% of the embryos were found to be atrophied *in utero*. This increase in atrophied embryos was remarkable. Histological observation of uteri showed endometric expansion and decrease in the lumen of uterus. Statistical analysis did not show significant variation in C. R. or body and placenta weight in non-atrophied embryos.

Discussion

Results obtained in this work agree with those arrived at in the works of Schou and Adisen [17], Smithberg [19] and Jurand [6] and show that lithium carbonate is a teratogenic drug and mainly neurotropic. The experiments revealed that lithium mainly affects the cranial and abdominal areas and specifically the skeletal system. IP application of a single dose 300 mg/kg of body weight on days 7.5, 8.5 and 9.5 of pregnancy in Balb/C mice produced eye defects, both unilateral and bilateral anophthalmia, and exencephaly. These findings agree partly with those of Jurand [6] who found exencephaly, cranioschisis, kinking of the spinal cord and dilation of the fourth brain ventricle. We did not find any endodermal organ defect, but there were various ectomesodermal organ defects. This is in agreement with the report of Wissooq *et al.* [23].

With regard to the transport of lithium through the placenta [16, 21, 24], it is postulated that lithium acts on the developing embryonic organs approximately six hours after the injections are administered.

A significant decrease in the C. R. and body weight ($P < 0.001$) was observed in the embryos of days 7.5 and 8.5 gestation. It is concluded that lithium affects the placental tissues and prevents normal development of the embryos. Atrophy was the most common effect in all experiments. On day 7.5, 5.7%; day 8.5, 34% and day 9.5, 50% atrophied embryos were observed, while none were found in the control group. We think this range of degeneration is due to embryonic age and susceptibility. In early developmental stages, the embryonic tissues have more resistance to the lithium, but as they age, this condition is reduced and the rate of atrophied embryos is increased.

Exencephaly was the other main defect (8% on day 8.5 of experiment). In this defect, most parts of the brain had developed outside the neurocranium, which was due to the stage of neural tube formation. We have neural fold stage on day eight which is a critical period for brain development. Our results agree with those of Jurand [6] who postulated that exencephaly is due to the defect in the process of the closure of the anterior part of the neural tube. Since lithium affects

Table 5. Number and percent of normal and atrophied embryos on day 9.5 of gestation

Observations	Number	Percent
Normal embryos	45	50
Atrophied embryos	45	50
Total	90	100

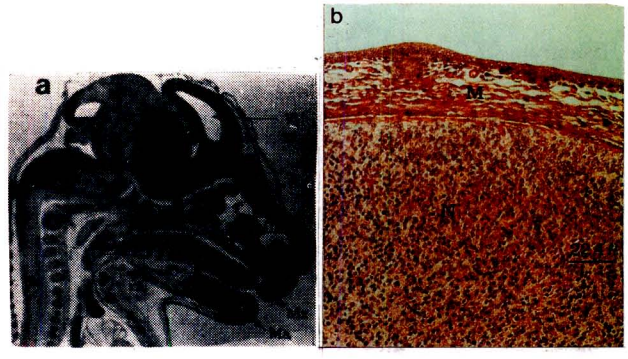


Figure 13. a) Stereophotomicrograph from the head region of control embryo on day 15.5 of gestation. X56.
b) Cross section of meninges of the same embryo. M, meninges X437

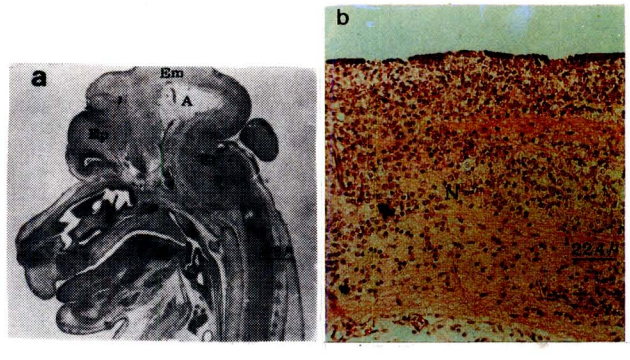
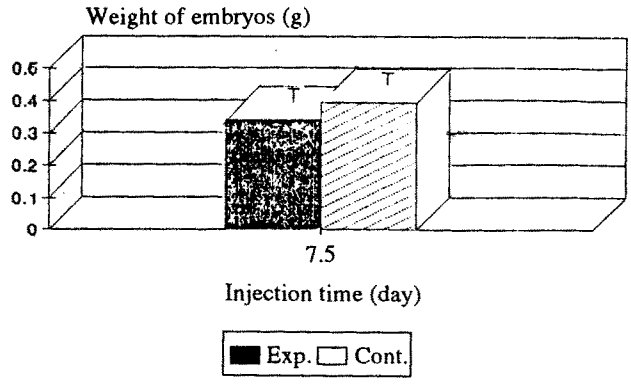


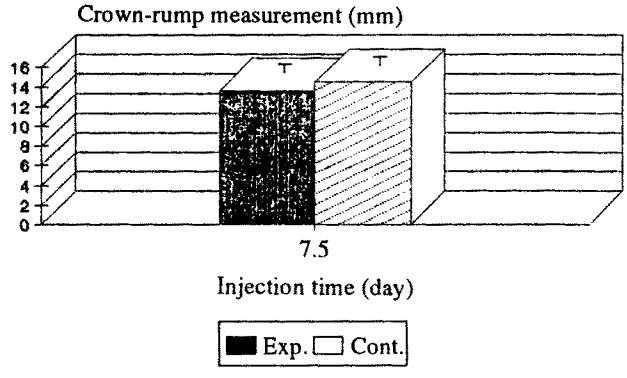
Figure 14. a) Stereophotomicrograph from the head region of experimental embryo with a total exencephaly treated on day 8.5 of gestation X56. b) Cross section of meninges region of the same embryo X437. Compare undeveloped meninges with control (Fig. 13b). A, arachnoid; C, cereberal hemispheres; Em, mesencephalon; Er, rhombencephalon; Ep, prosencephalon; Ma, mandible, Me, meninges; Mx, maxilla; N, nerve tissue; Sc, spinal cord; Tu, tongue; Vi, lateral ventricle; V3, 3rd ventricle.

Histogram 1. Comparison between weight of control and experimental embryos on day 15.5 of gestation



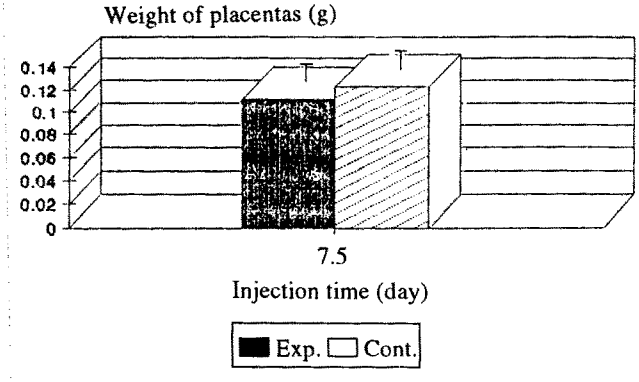
The experimental mice have been injected on day 7.5 of gestation.

Histogram 2. Comparison between crown-rump of control and experimental embryos on day 15.5 of gestation



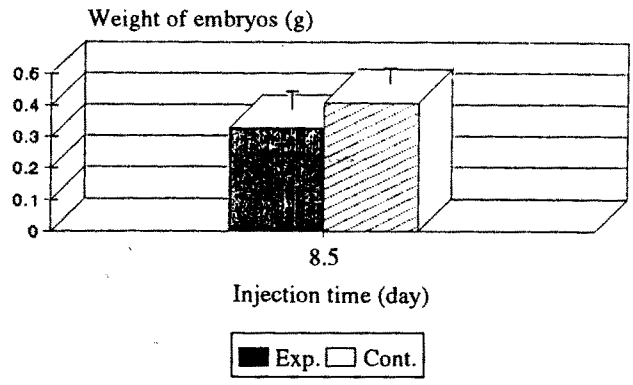
The experimental mice have been injected on day 7.5 of gestation

Histogram 3. Comparison between weight of control and experimental placentas on day 15.5 of gestation



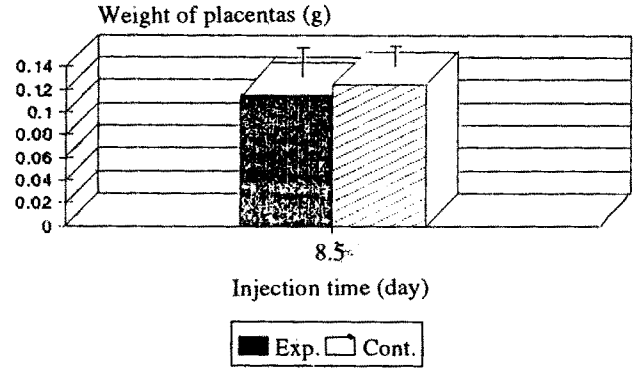
The experimental mice have been injected on day 7.5 of gestation.

Histogram 4. Comparison between weight of control and experimental embryos on day 15.5 of gestation



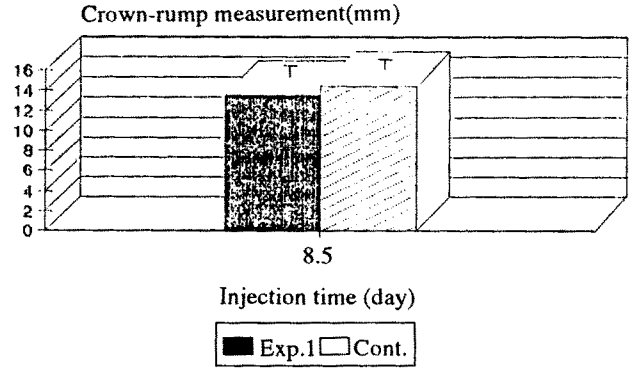
The experimental mice have been injected on day 8.5 of gestation.

Histogram 5. Comparison between weight of control and experimental placentas on day 15.5 of gestation



The experimental mice have been injected on day 8.5 of gestation.

Histogram 6. Comparison between crown-rump of control and experimental embryos on day 15.5 of gestation



The experimental mice have been injected on day 8.5 of gestation.

the target organ such as neural tissues after 4-8 hours injection, it can be concluded that lithium affects the tube formation and morphogenic cell movements during days 8.5 - 9, that is to say the brain tissues do not approach their normal sites. Sagital sections of the exencephalic foetuses showed two distinct mal-developments:

1. Meninges in parts of exencephalic brain tissues had not been developed.
2. Visceral bones of the skull did not show normal orientation. Results of both unilateral and bilateral anophthalmia defect did not show any differentiation of eye tissue, this is due to the effect of lithium during the 7.5-8 day period which is a critical period in optic vesicle and eye cup development.

Lithium has blocked inductive agents of three consecutive induction stages in the eye development (optic cup, lens and cornea formation). In microphthalmic embryos, the lens appeared smaller than the normal size and had no fibrillation in the cells, disorganization in the retina was also observed. This may be due to the incomplete induction and development of optic and lens vesicles and abnormal migration of head mesenchymal cells. The mechanisms of less frequent defects such as cleft palate, coelosomy and partial exohepaty remain obscure in this work.

At the cellular level, it seems that lithium is transported through Na^+ - Li^+ co-transport system, in other words, lithium uses the same transporting mechanism as sodium does. One of the most characteristic actions of lithium is suppressing the metabolism of inosithol phosphate [1] as an incompetent for metabolism of inositol. That is, the ion blocks hydrolysis of intermediate inosithol phosphates and stops their recycling. On the other hand, works on the effect of lithium on the catalytic action of adenylate cyclase of rat brain tissues have shown that this ion blocks this enzyme [13] and it may also affect the calmodulin-enzyme interactions or substrate with divalent ions as well.

In conclusion it can be postulated that lithium disorganizes the second messenger system (Ca^{++} - calmodulin and phosphatydil inositol) and causes considerable defects in embryonic development.

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