



## EFFECT OF ACTINOMYCIN D AND LITHIUM CHLORIDE ON CHICK EMBRYO CULTIVATED IN VITRO

### Zoology

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### ABSTRACT

The effect of 0.05 µg/ml Actinomycin D (an inhibitor of protein synthesis) and 0.025M Lithium chloride (inhibitor of glycogen synthase activity or inositol triphosphate) have been studied on chick embryo cultivated in vitro. The eggs were incubated at 37.5°C for 18 hours to obtain stage 4 embryo (primitive streak stage) for Actinomycin D treatment & for Lithium chloride incubation was done for 24-33 hrs. Embryos were again incubated for 20 hrs. by using New's ring technique. The result shown that the effect of Actinomycin D & Lithium chloride causes different developmental abnormalities viz. abnormal heart formation, stunted or bent axis, deformed somites abnormal neural plate formation. It is concluded that Actinomycin D is the teratogen which binds to DNA duplex thereby interfering with the enzymes engaged in replication & transcription. It also inhibits AMP-dependant changes to protein synthesis. Lithium chloride induces malformation in chick embryo similar to Actinomycin D. It inhibits mitotic activity in chick embryo. It mostly affects the activities of somites as well as normal heart & head formation.

### KEYWORDS

Chick embryo, Actinomycin D, lithium chloride, teratogen

### INTRODUCTION

Actinomycin D in Various embryonic systems shows the teratogenic effects. The malformation in amphibian's system and chick embryos which involved the nervous system, the eyes and cardio vascular system etc. are caused by teratogenic effects of Actinomycin D.

In the present work, it is seen that Actinomycin D causes microcephaly in 60% of chick embryos treated as primitive streak stage (stage 4), inhibits the formation of somites and heart in 63% and 80% cases respectively. In some cases, shortening of axis is also observed. The embryos which are treated with Actinomycin D at the head process stage (stage V) in which anterior structures are already determined show abnormalities of posterior axis in dead somites formation through abnormal is not drastically affected (Hamburger and Hamilton) to differential response in stage 4 and stage 5 towards toxicity of Actinomycin D suggest that Actinomycin D primarily affects biosynthesis of the new nucleic acids rather than their functioning. Actinomycin D is known to exert a performed effects on cellular nucleic acids and it interference with DNA dependent RNA polymerase enzyme (Reich et al). Recently it has been suggested that Actinomycin D affects cell division and did not induces sister chromatid exchange in Euglena.

The teratogens in early chick embryo have been also explored using lithium chloride (LiCl). Lithium Chloride is used to treat depression in adult, causes a very small (<0.1%) increase in proportion of birth defects in humans. It interferes with the normal global patterning of very early in fish, frog and chicks. It causes dorsalization in all embryos in all these embryos. Lithium Chloride treatment of chick should be continuous, 0.3 M LiCl added to blastoderm in egg shell is a good starting point. LiCl inhibit the cell population growth, epiboly and shaping of organ primordial. In most abnormal embryos the cell population size and blastoderm area are inhibited most.

LiCl is the known teratogen that has been known to increase the proportion of sea urchin embryo cells that contribute to the archenterons. It has shown to cause major morphological changes I sea urchin, chick and mouse embryo development, specially, affecting the veg1 cell lineage [6]. LiCl has been proven to cause abnormality exaggerated development of structures derived from vegetal area. It also results in formation of embryos with proportionately large archenterons or even with a archenterons that buldges outward from the surface invagination properly in to the blastocoel. The teratogenic effect of LiCl on embryo on development is likely to do mediate through specific inhibition of the activity of either glycogen synthase kinase (Klein and Melton, 1996) or inositol triphosphate (Berridge, 1993). LiCl is an antidepressant and may affect the central nervous system. It is now shown that during development, cell proliferative activity and area expansion are dissociated in time and the extent of abnormal development induce by LiCl is directly co-related with a

decreased proliferative activity and blastoderm expansion.

### MATERIALS AND METHODS

#### MATERIALS:

Fresh and fertilized hen's egg, Pc saline, 0.05 g/ml solution of Actinomycin D, 0.025 M solution of LiCl, petriplates, watch glasses, glass ring, cotton rings, flasks (2000 ml, 500 ml) cake dish, scissor, binocular, eosin, hematoxyline stain, etc.

#### Preparation of Pc Saline (Panette Compton Saline):

- Solution A:** Dissolve 24.22 grams NaCl, 3.1 grams KCl, 2.54 grams MgCl<sub>2</sub>, 3.08 grams CaCl<sub>2</sub> in 200 ml distilled water one after another.
- Solution B1:** Dissolve 1.875 grams Na<sub>2</sub>HPO<sub>4</sub> in 360 ml distilled water.
- Solution B2:** Dissolve 0.08 grams NaH<sub>2</sub>PO<sub>4</sub> in 40 ml distilled water.
- Glucose Solution:** Dissolve 13.5 grams glucose in 1350 ml distilled water.

Mix 8 ml of B2 solution + 88 ml of B1 solution = 96 ml solution B.

Solution A (60 ml) + Solution B (90 ml) + Glucose solution (1350 ml).



1500 ml Pc Saline.

#### Preparation of Bouin's Fixative:

Dissolve 75 ml picric acid (saturated sol) in 25 ml 40% formaldehyde and add 5 ml Glacial acetic acid to form 105 ml Bouin's Fixative.

#### METHOD:

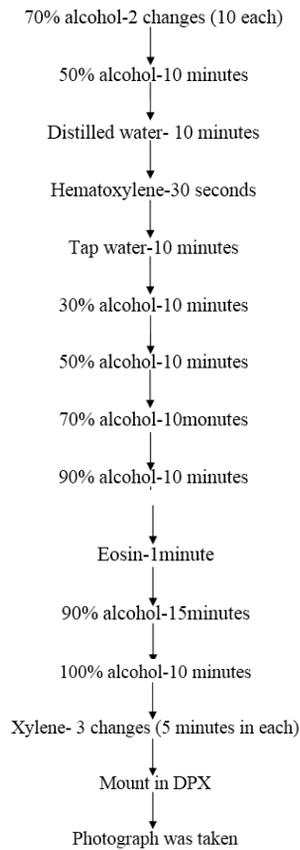
- Eggs were incubated at 37.5°C for 18 hours. To obtain stage 4 embryo (primitive stage) for Actinomycin D treatment & for LiCl treatment incubation was done for about 24-33 hours.
- Glassware's employed in the experiment were sterilized and culturing solutions were autoclaved.
- Embryos were cultured using New's ring technique.
- New's ring technique:
- Break the egg shell by using blunt forceps at the blunt end of egg.
- Discard all the chick albumen and collect thin albumen in a small beaker.
- Yolk ball is completely immersed in cake dish containing Pc Saline and cut the vitelline membrane along the equatorial position.
- Separate the vitelline membrane from the yolk ball spread over the glass in such a way that ventral side faces upwards.
- Wash the membrane with the help of bent pipette containing Pc saline.

Glass ring is placed over the vitelline membrane. Remove all the Pc

- Saline outside the ring and transfer the watch glass into another petriplate containing cotton ring.
- Add 5-6 drops of Pc Saline inside ring and thin albumen outside the ring (For control).
- Treat the embryos with 0.05 mg/ml Actinomycin D solution/ 0.025 LiCl solution for experimental purpose.
- Care was taken that Actinomycin D and LiCl added gently by the side of blastoderm so that it is evenly exposed to antibiotics.
- Incubate the embryos at 37.5°C for about 20 hours.
- Fix the embryos in Bouin's fixative, keep it for 20 hours then store in 70% alcohol.

**WHOLE MOUNT**

For the whole mount eosin and hematoxyline stains were used. For the whole mount following steps were involved.



**RESULTS AND DISCUSSIONS**

**Part I: Effect of Lithium Chloride on Chick embryo Observations and Results**

**Table I:** Total number of abnormal embryos in Actinomycin D treatment

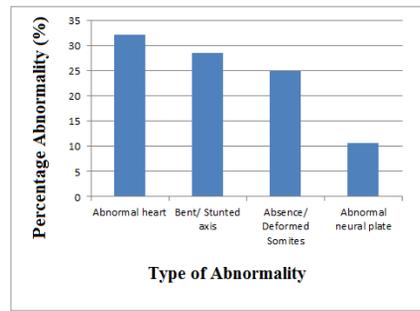
QUANTITY OF EGGS	NO. OF CONTROL EMBRYOS	NO. OF EXPERIMENTAL EMBRYOS	NO. OF ABNORMAL EMBRYOS
11	03	08	06
10	03	07	07
10	03	07	06
12	03	09	08
<b>TOTAL</b>			
43	12	31	27

**Table II:** Individual abnormality percentage in LiCl treated embryos

Sr. No.	No. of Embryos	Abnormalities	% individual abnormality	Total abnormality
1.	09	Abnormal heart	32.1	87
2.	08	Bent/ Stunted axis	28.5	

3.	07	Absence/ Deformed Somites	25.0	
4.	03	Abnormal neural plate	10.7	

**GRAPH: ABNORMALITY PERCENTAGE VS TYPE OF ABNORMALITY**



**Fig. 1:** 33 hours chick Embryo served as a Control



**Fig.2:** 27 Hours Chick Embryo served as a Control



**Fig.3:** Entire Chick Embryo treated with Actinomycin D at primitive Streak Stage

- Abnormal Somites, Abnormal heart & Neural Plate
- Bent Axis



**Fig.4:** Entire Chick Embryo treated with Actinomycin D at primitive streak stage



**Fig.5: Entire Chick Embryo treated with Actinomycin D at primitive streak stage**

- Stunted growth, Bent axis



**Fig.6: Entire Chick Embryo treated with Actinomycin D at Head Process stage (Stage 5)**

- Bent axis, Somites abnormality, inhibition of Heart Formation.

**CONCLUSIONS**

The Actinomycin D molecule consists of Phenoxazone ring system. It is excluded by viable cells but can penetrate the cell membrane of dead cells. Actinomycin D is the teratogen which binds to DNA duplex there by interfering it with the action of enzymes engaged in replication and transcription. It affects the biosynthesis of new nucleic acid rather than their functioning. it complexes with DNA and also prevents synthesis of RNA. Presence of Actinomycin D inhibits cAMP- dependent changes protein synthesis. The stimulation of NAT (Serotonin N acetyl transference-regulatory enzymes in melatonin biosynthesis pathway was examined in monolayer cultures of chick retinal cells) activity was inhibited by Actinomycin D.

It is also shown that Actinomycin d have a potent inducers of apoptosis. It causes excephaly, anophthalmia, microphthalmia, spina bifida in rat.

**Part II: Effect of Lithium Chloride on Chick embryo Observations and Results**

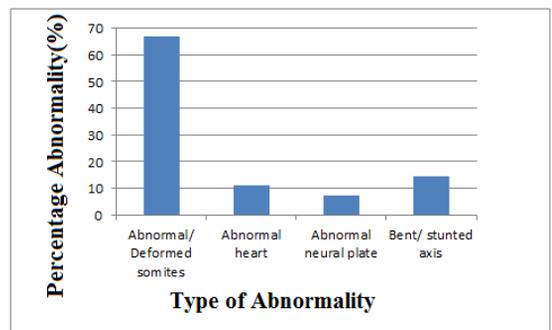
**Table III:** Total number of abnormal embryos in LiCl treatment

QUANTIT Y OF EGGS	NO. OF CONTR OL EMBRY OS	NO. OF EXPERIM EN TAL EMBRY OS	NO.OF ABNORM AL EMBRY OS	STAGE
08	02	06	06	P.S, 3,4,9,10. SOMITES STAGE
15	06	09	09	4,5,6,10 SOMITES STAGE
11	02	09	09	4,5,6,10 SOMITES STAGE
04	01	03	03	P.S. & 4 SOMITES STAGE
<b>TOTAL</b>				
38	11	27	27	

**Table IV:** Individual abnormality percentage in LiCl treated embryos

Sr. No.	No. of Embryos	Stage Abnormalities	Abnormalities	% individual abnormality	Total abnormality
1.	18	P.S. 3,4,5,6,8,9 & 10 somites stage	Abnormal/ Deformed somites	66.6	100
2.	03	P.S. 5,9 & 10 somites stage	Abnormal heart	11.1	
3.	02	P.S. 5,9 & 10 somites stage	Abnormal neural plate	7.4	
4.	04	P.S. 4,6 & 9 somites stage	Bent/ Stunted axis	14.8	

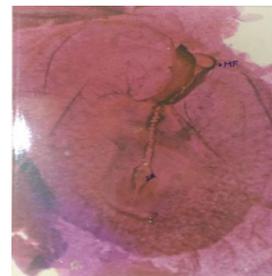
**GRAPH II: ABNORMALITY PERCENTAGE VS TYPE OF ABNORMALITY**



**Fig.7: 33 hours chick Embryo served as a control**



**Fig.8: Whole Mount of Chick Embryo at 15-16 somites stage served as a control**



**Fig. 9: Entire Chick Embryo treated with Lithium Chloride at 24 hours Stage (4 somites)**

- Complete absence of somites, abnormal neural plate formation stunted axis



**Fig. 10:** Entire Chick Embryo treated with lithium chloride at 24 hours (4 somites stage)

- Abnormal neural plate formation and stunted axis



**Fig. 11:** Entire Chick Embryo treated with Lithium chloride at stage 6 (24 hours embryo)

- Abnormality in neural plate and neural fold, Heart shifting and slightly bending of axis



**Fig. 12:** Entire Chick Embryo treated with Lithium Chloride at 4 somites stage (24 hours)

- Abnormal Head formation, Heart Shifting

## CONCLUSIONS

Lithium Chloride induces malformation in chick embryo similar to Actinomycin D. LiCl inhibit normal morphogenesis in chick embryo, suggested that it inhibit the mitotic activity in chick embryos. It inhibits the cell population doubling time and suppresses the normal cell cycle [13].

Lithium, as well known teratogan affects cell fate determination in sea urchin embryos by causing exaggeration of vegetally derived tissues [6].

A long term treatment of LiCl on pure culture of chicken neurons decreases the serotonin and increases the uptake of norepinephrine [15]. Lithium interference with correct folding of tubulin polypeptides normally hidden to the action of the protease. LiCl treatment on chick embryo mostly affects the activity somities as well as normal heart and head formation.

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