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Effect of lithium on rat embryos in culture: growth, development, compartmental distribution and lack of a protective effect of inositol

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Abstract. Lithium chloride (LiCl) was tested at various concentrations (50, 100, 150 and 200 µg/ml) using a rat whole-embryo culture system beginning on gestation day 9.5 (headfold stage) in order to establish a concentration-response relationship. Open neural tubes – as described in former publications (Tesh 1988) – were not induced by lithium. A significant reduction of embryonic growth and development occurred at the lowest concentration tested (50 µg/ml). Clear-cut dysmorphogenetic effects (absence of the eye cup, kink in the spinal anlage, "blebs" at the rostral head region) occurred at a concentration of 150 µg/ml LiCl. LiCl concentrations in the embryo, visceral yolk sac, exocoelomic fluid and medium were determined after the embryos had been cultured for 48 h in the presence of a moderately embryotoxic dose of LiCl (3.5 mEq/l or 150 µg/ml). Medium supplementation with *myo*-inositol in different concentrations was ineffective in antagonizing the embryotoxicity induced by LiCl.

Key words: Whole-embryo culture – Lithium caused embryotoxicity – No protective effect of *myo*-inositol

Introduction

Lithium is utilized for treatment of the manic phase of bipolar depression (Rana and Hokin 1990). Because women of child-bearing age can develop these symptoms, there is the potential for exposure during pregnancy. The human dose is titrated so that plasma lithium levels of approximately 0.8–1.2 mM are maintained during therapy 10–12 h following the last oral administration (Ehrlich and Diamond 1980). Serious toxic side-effects are expected to occur at plasma levels greater than 2 mM.

Lithium crosses the human placenta (Rane et al. 1974) and lithium administration has been associated with several congenital anomalies (reviewed by Warkany 1988). Most attention has focussed on Ebstein's anomaly which is a downward displacement of the tricuspid valve which leads to an incorporation of the right ventricle into the right atrium. Recent evidence indicates that the association of lithium use during pregnany and the induction of Ebstein's anomaly is, if it exists at all, not strong (Kallen 1988; Edmonts and Oakley 1990). Jacobson et al. (1992) concluded that there is no indication that lithium is an important human teratogen.

The biochemical mechanisms of lithium action which are responsible for the therapeutic as well as possible adverse effects are largely unknown. However, it is well established from in vivo studies that lithium inhibits the hydrolysis of inositol phosphate to free inositol which is required for the regeneration of phosphatidyl inositol in membranes (Drummond 1987). It has been hypothesized that the teratogenic effects of lithium may also be due to inhibition of the inositol phosphate metabolism (Berridge 1986). Inositol can protect *Xenopus* embryos from alterations in pattern formation which are caused by lithium. This protective response is specific for *myo*-inositol and no protection is afforded by *epi*-inositol (Busa and Gimlich 1989).

Investigations using rat whole-embryo culture have demonstrated that the absence of *myo*-inositol in the medium is specifically associated with an open cranial neuropore in the region of the hindbrain (Cockroft 1979, 1988). If the effect of lithium is merely to prevent the regeneration of inositol from phosphorylated precursors, then lithium should prevent anterior neural tube closure. We have tested this hypothesis using the rat whole-embryo culture, and we also attempted to clarify diverging reports of effects of lithium on development in this system.

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Table 1. Concentration-effect relationship of LiCl

	Yolk sac (mm)	Crown-rump length (mm)	Somites (n =)	Protein (µg/E)	Score	%Abn[a]	%Abn[b]
	5.19	4.13	29	245	38		
Control	4.68	3.84	28	222	38	–	–
	4.32	3.74	27	209	37		
n = 16							
	4.74	3.96	28	257	37		
50 µg/ml	4.62	3.72*	27*	194	36**	–	–
	4.35	3.54	26	184	36		
n = 21							
	4.68	3.83	28	212	35		
100 µg/ml	4.47	3.48**	27*	189*	34**	–	–
	4.32	3.38	26	175	33		
n = 20							
	4.61	3.54	28	163	33		
150 µg/ml[c]	4.32*	3.36**	27*	151**	31**	25	60
	3.98	3.02	25	136	29		
n = 20							
	4.38	3.12	25	125	28		
200 µg/ml	3.96**	2.82**	24**	101**	23**	84	100
	3.78	2.70	23	84	21		
n = 19							

In this table median values are given; bottom and upper numbers represent the first and the third quartile

* significantly different ($0.01 < p < 0.05$) (Mann-Whitney test) from controls

** significantly different ($p < 0.01$) (Mann-Whitney test) from controls

[a] abnormalities excluding absence of the eye cup

[b] absence of the eye cup

[c] 150 µg/ml or 3.5 mEq/l

[abn] frequency of the abnormalities in %

Materials and methods

Animal maintenance and mating procedure. Wistar rats (Bor: Wisw/spf, TNO; Winkelmann, Borchen, FRG) were kept under spf conditions at a constant day/night cycle (light from 9.00 a. m. to 9.00 p. m.). One male was caged together with three female animals for a mating period from 6.00 a. m. to 8.00 a. m. and the following 24 h were called day 0 of pregnancy if sperm were detected in the vaginal smears.

Test substances. Lithium was purchased from Fluka Chemie AG (CH-9470 Buchs) as lithium chloride anhydrous. *Myo*-inositol was supplied by Sigma Chemie GmbH, D-8024 Deisenhofen. Both compounds were dissolved in Tyrode's buffer to obtain different stock solutions. To reach the concentrations of the compounds finally tested in the culture medium 1 ml or 0.5 ml (addition of inositol and LiCl) of the corresponding stock solution was added to 6 ml bovine serum.

Whole-embryo culture. A modified technique for cultivation of early postimplantation rat embryos in a roller culture system – as developed by New and coworkers (Cockroft 1977; New 1978) – was applied. The modified experimental and evaluation conditions have been described in detail before (Klug et al. 1985). Heat-inactivated and sterile-filtered bovine serum (6 ml), supplemented with Tyrode's buffer (1 ml) enriched with 525 µg methionine/ml, served as culture medium for three to four 9.5-day-old embryos (headfold stage, 3–4 somites) per culture flask. The embryos were cultured for 48 h at 38.5° C in a roller device (25 rpm). Initially, the bottles were gassed with 10% O_2, 5% CO_2 and 85% N_2, after 36 h the O_2 concentration was raised to 50% (5% CO_2, 45% N_2). At the end of the culture period the embryos were evaluated with regard to growth and differentiation using a scoring system similar to that of Brown and Fabro (1981).

Pharmacokinetic analysis. Separate experiments were performed to measure the LiCl concentration in the yolk sac, the exocoelomic fluid and the embryonic tissues. The embryos were exposed to 150 µg LiCl/ml culture medium for 48 h and were then removed from the culture flask, washed in 0.9% NaCl solution and transferred to a siliconized Petri dish where the remaining washing fluid was blotted away with filter paper wedges. The exocoelomic fluid was collected by insertion of a hypodermic needle into the corresponding cavity. The remaining material was separated into embryos and yolk sac. The material from each experiment was pooled and stored frozen after determining the sample weight.

The pooled embryos and membranes were homogenized by ultra sonication in 1 ml of 0.9% NaCl solution. The same amount of NaCl was added to the exocoelomic fluid samples where no NaCl was added to culture medium samples. All samples were then diluted 1:50 with a solution containing: 0.64 g CsCl and 0.1 N HCl to 5 l. Analyses were performed with a flame spectrophotometric system (Carl Zeiss, D-7082 Oberkochen, Germany; Model Elektrolytautomat FL 7) and results expressed as LiCl in mmol/l.

Histological examination. At the end of the culture embryos from each group were fixed in Bouin's solution and embedded in Paraplast[R] (Monoject Scientific Inc., Kildare, Ireland). Sagittal and horizontal serial sections were cut, stained with hematoxylin/eosin and photographed and evaluated.

Results

Concentration-effect relationship of LiCl

The morphological data describing the concentration-effect relationship are given in detail in Table 1. The typical morphological outcome after exposure to LiCl is illustrated in Fig. 1.

Addition of 50 µg LiCl/ml culture medium reduced growth (crown-rump length, protein content) and differentiation (somites, morphological score) of the cultured

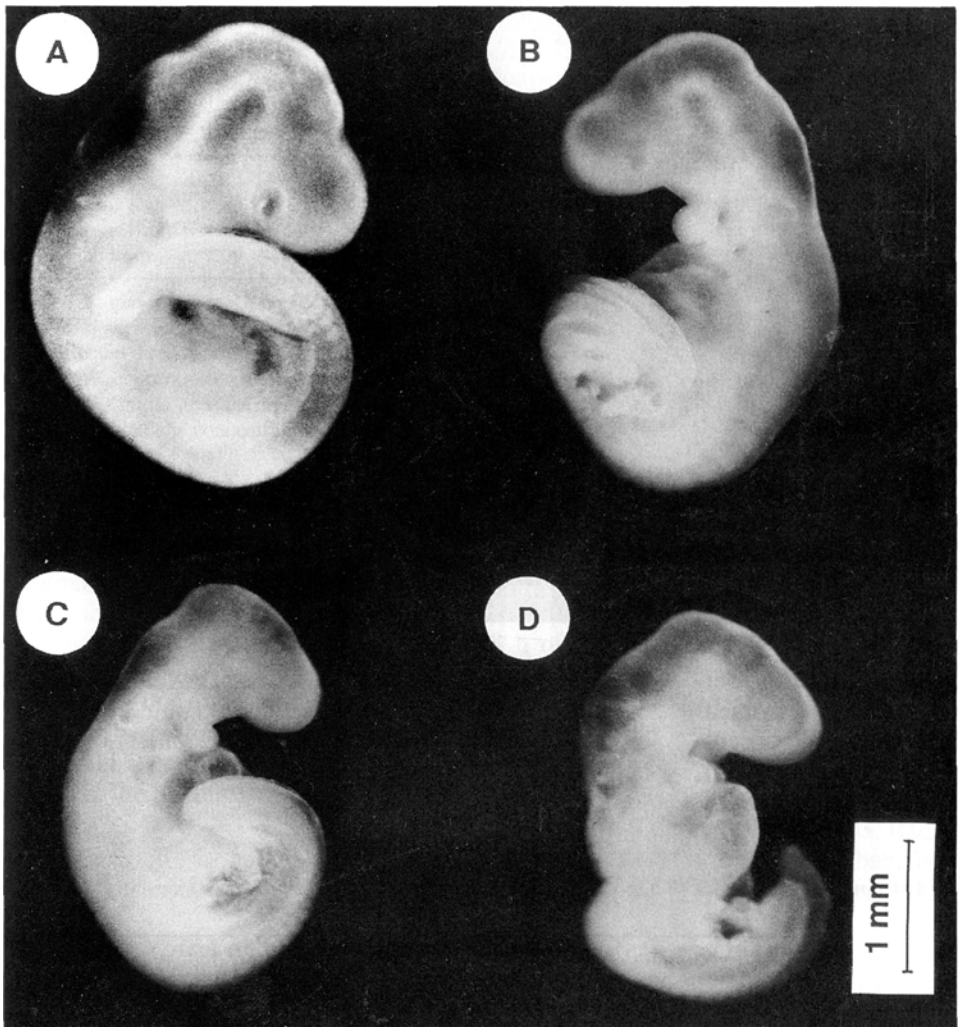

Fig. 1. Development of 9.5-day-old rat embryos after 48 h in culture. **A** Control, **B** 100 µg LiCl/ml, **C** 150 µg LiCl/ml, **D** 200 µg LiCl/ml

embryos. This is apparently a "proportional retardation", since growth and differentiation were affected in parallel. The significant reduction of the total score is due to a delayed development of the fore- and hindlimbs.

At 100 µg LiCl/ml the degree of retardation was more pronounced and additional organ anlagen were affected: the ear anlage was partially open and the recessus dorsalis was not developed. Moreover, the tailbud was shortened in comparison to the controls. Nevertheless, the deviations from normal development at this concentration of LiCl were considered as retardations rather than gross-structural abnormalities.

An increase of LiCl to 150 µg/ml caused clear-cut dysmorphogenesis (gross-structural abnormalities): 25% of the cultured embryos showed either "blebs" at the rostral head region and/or a kink in the spinal anlage ("retractions"). In addition to the retardation observed with lower LiCl concentrations, all embryos had open ear vesicles and frequently the heart anlage did not show the typical "sigmoidal" flexure. The most frequent adverse effect concerned the development of the eye: *60%* of the embryos cultured at this concentration showed no formation of the eye cups.

LiCl concentrations of 200 µg/ml did not lead to additional types of defects, but only the degree and frequency of the observed effects (retardation, abnormalities) increased. None of the cultured embryos showed formation of the eye cup.

The development of the yolk sac was not affected when the cultured explants were exposed to 50 or 100 µg LiCl/ml. At the higher concentration tested a clear-cut pathological development was noted: in the presence of 150 µg LiCl/ml 50% of the embryos showed a severely disturbed yolk sac circulation. The majority of the yolk sac vessels were not linked, the yolk sac resembled a "golf ball" (cf. Fig. 2) and the blood circulation appeared to be non-functional. These effects occurred at a 100% incidence when the embryos were exposed to 200 µg LiCl/ml.

Effect of LiCl (150 µg/ml) in culture medium supplemented with inositol

The data are compiled in Table 2. The control group consisted of all embryos cultured with varying concentrations of inositol only (3.5–56 mM). There was no difference in embryonic development when cultured with these widely differing concentrations of inositol.

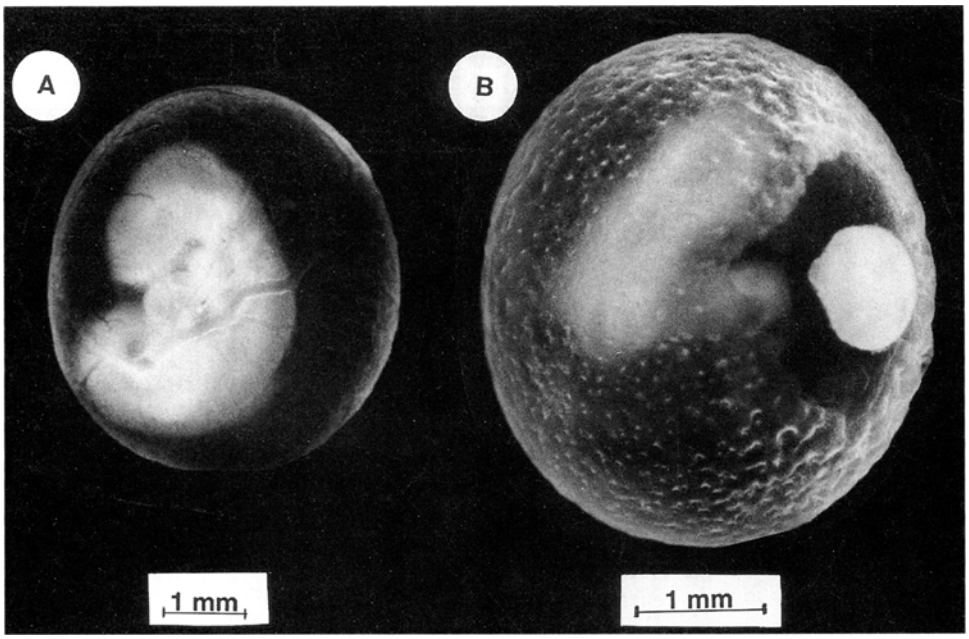

Fig. 2. Development of 9.5-day-old rat embryos after 48 h in culture: yolk sac development. **A** Control, **B** 200 µg LiCl/ml

The effect observed with 150 µg LiCl/ml was not changed when the culture medium was supplemented with 3.5 mM or 7 mM inositol. At higher inositol concentrations (10.5–56 mM) the LiCl effect was augmented: 50–60% of all embryos showed gross-structural deviations of the shape and the head as described above. No influence of inositol (either positive or negative) could be observed on the LiCl-induced effect on the development of the eye cup.

Table 2. Growth and development of 9.5-day-old rat embryos exposed for 48 h to 150 µg LiCl with increasing supplementation of inositol to the culture medium

	Yolk sac (mm)	Crown-rump length (mm)	Somites (n =)	Protein (µg/E)	Score	%Abn
Inositol	4.74	3.90	28	250	38	
only	4.62	3.72	28	218	38	7
	4.44	3.66	27	173	35	
n = 44						
+3.5 mM	4.80	3.54	28	180	36	
Inositol	4.35	3.30**	26**	128**	34**	20
	4.17	3.05	25	104	30	
n = 30						
+7 mM	4.68	3.48	24	153	30	
Inositol	4.44	3.27**	26**	129**	32**	16
	4.07	3.00	25	101	30	
n = 30						
+10.5 mM	4.64	3.18	24	150	30	
Inositol	4.44	3.12**	24**	130**	29**	58
	4.32	3.02	22	106	28	
n = 12						
+28 mM	4.44	3.42	27	132	31	
Inositol	4.14**	3.24**	26**	117**	28**	55
	3.90	2.97	25	95	25	
n = 9						
+56 mM	4.11	3.14	26	101	29	
Inositol	3.99**	3.12**	26**	86**	29**	50
	3.87	3.00	25	70	27	
n = 10						

In this table median values are given; bottom and upper numbers represent the first and the third quartile

* = significantly different ($0.01 < p < 0.05$) (Mann-Whitney test) from controls

** = significantly different ($p < 0.01$) (Mann-Whitney test) from controls

%Abn = frequency of the abnormalities in %

Table 3. Lithium concentrations in culture medium and embryonic compartments 48 h after the initiation of culture in 150 µg LiCl/ml culture medium (= 3.5 mEq Li^+/l)

Samples	Number of samples[a]	Li^+ concentration (mEq/l)[b] (mean ± standard deviation)
Embryos	3	1.51 ± 0.51
Visceral yolk sac	3	1.09 ± 0.22
Exocoelomic fluid	3	1.56 ± 0.13
Culture medium	9	3.28 ± 0.19

[a] Each sample represents a pooled collection of the appropriate tissue or fluid from approximately 20 embryos except the culture medium which was collected directly from a bottle in which four embryos were cultured
[b] Lithium concentration measurements were performed in triplicate from each sample and the mean value of the three measurements was used to calculate the concentration

Concentration of LiCl in the embryonic compartments

Table 3 gives the LiCl concentrations determined by flame photometry for the embryo, the yolk sac, the exocoelomic fluid and the culture medium following 48 h of culture. The concentration in the embryo was approximately the same as in the exocoelomic fluid and this was roughly one-half the culture medium concentration. The yolk sac LiCl level was found to be about one-third of the concentration in the medium.

Histological evaluation

There was no great difference in the morphological effect between the two concentrations (150 and 200 µg/ml LiCl) which induced significant defects during development as judged by light microscopy. All embryos showed a loosening of the mesenchyme and the occurrence of large and occasionally huge cavities in these areas that were often delineated by an endothelium-like thin cellular layer; other wall structures were missing. Blood cells were visible in some of these cavities which were therefore considered as sinus-like, embryonic vessels. However, their size and location clearly differed from the vascular system of untreated controls. The loosening and thinning out of the mesenchyme occasionally reached such an extent that especially between neuroepithelium and outer ectodermal epithelium in the head region, mesenchymal cells could often no longer be observed (Figs. 3, 4).

The size of the somites in the same embryo could vary considerably. Aggregation and adhesion of the cells were not obviously disturbed. Even the smallest somites exhibited an epitheloid arrangement of the cells. The size of the somites, however, varied considerably. An explanation might be an insufficient migration of presomitic cells towards the later somites.

Alterations in the mesenchyme were also seen in the region of the heart anlage (Fig. 5). Under normal conditions cells migrate during the culture period from the outer myoepithelial coat and the inner endothelium to establish the typical heart mesenchyme (cardiac jelly) between these two layers. Such migration processes could not be demonstrated after exposure to LiCl. The result was wide spaces devoid of any cells. The morphology of the in- and outflow tracts was not damaged.

In the region of the brain anlage development and formation of the eye process were clearly disturbed. In the control embryos the invagination of the eye cups was well underway at this stage. However, LiCl exposed embryos had no eye cups (Fig. 4). In addition, growth of the optic process was obviously also inhibited. In lateral sagittal sections of the head, cross-sections of the optic process were often missing. This finding is due to a great distance between the tip of the optic process and outer epithelium of the embryonic skin. Horizontal sections confirmed this result. Interestingly, the previously described, large, wide-luminal vessels were also often observed around the optic process. Finally, the irregular contour of the endodermal tube should be mentioned. This contour was often found to be deformed in a saw-blade manner.

Exposure to different concentrations of inositol in the medium did not change the light microscopic appearance. A difference between the two treatment groups (LiCl alone or in combination with inositol) could not be established.

Discussion

There is a long history of publications describing the teratogenic effects of lithium in embryos of many species (Morgan 1903; Backstrom 1954; Herbst 1982). Teratogenic responses have been demonstrated in some species after lithium administration during the organogenesis stage but do not occur consistently. In mice the malformation pattern induced included cleft palates, skeletal anomalies and exencephaly (Szabo 1970; Wright et al. 1970; Loevy 1973; Smithberg and Dixit 1982; Jurand 1988). However, no teratogenic response to lithium in mice was reported in various other studies (Bass et al. 1951; Tuchmann-Duplessis and Mercier-Parot 1973). In rats there have been conflicting reports of lithium-induced developmental toxicity (Trautner et al. 1958; Johansen and Ulrich 1969; Gralla and McIlhenny 1972; Tuchmann-Duplessis and Mercier-Parot 1973). It has been suggested that the different findings in rats may result from strain differences (Hansen et al. 1990) since similar exposure regimens produced teratogenic effects in Sprague-Dawley rats (Wright et al. 1971), but not in Wistar rats (Johansen 1971).

The present study has demonstrated that whole rat embryos maintained in culture with LiCl for 48 h responded with a concentration-related degradation of growth and differentiation (Table 1). The types of defects seen were "blebs" at the rostral head region and/or kinking of the spinal anlage. Developmental retardation was detected in the limb buds, ear vesicles, tailbud, heart and the eye cup.

Previous studies of lithium effects on cultured rat embryos have reported different results as regards neural tube closure. Tesh (1988) described both cranial and caudal open neural tubes. Hansen et al. (1990) found a single open neural tube among 144 rat embryos when the culture period was initiated on day 10 of gestation. Day 10 of gestation in the rat is less sensitive to the induction of neural tube defects than day 9, i.e. day 10 might be too late

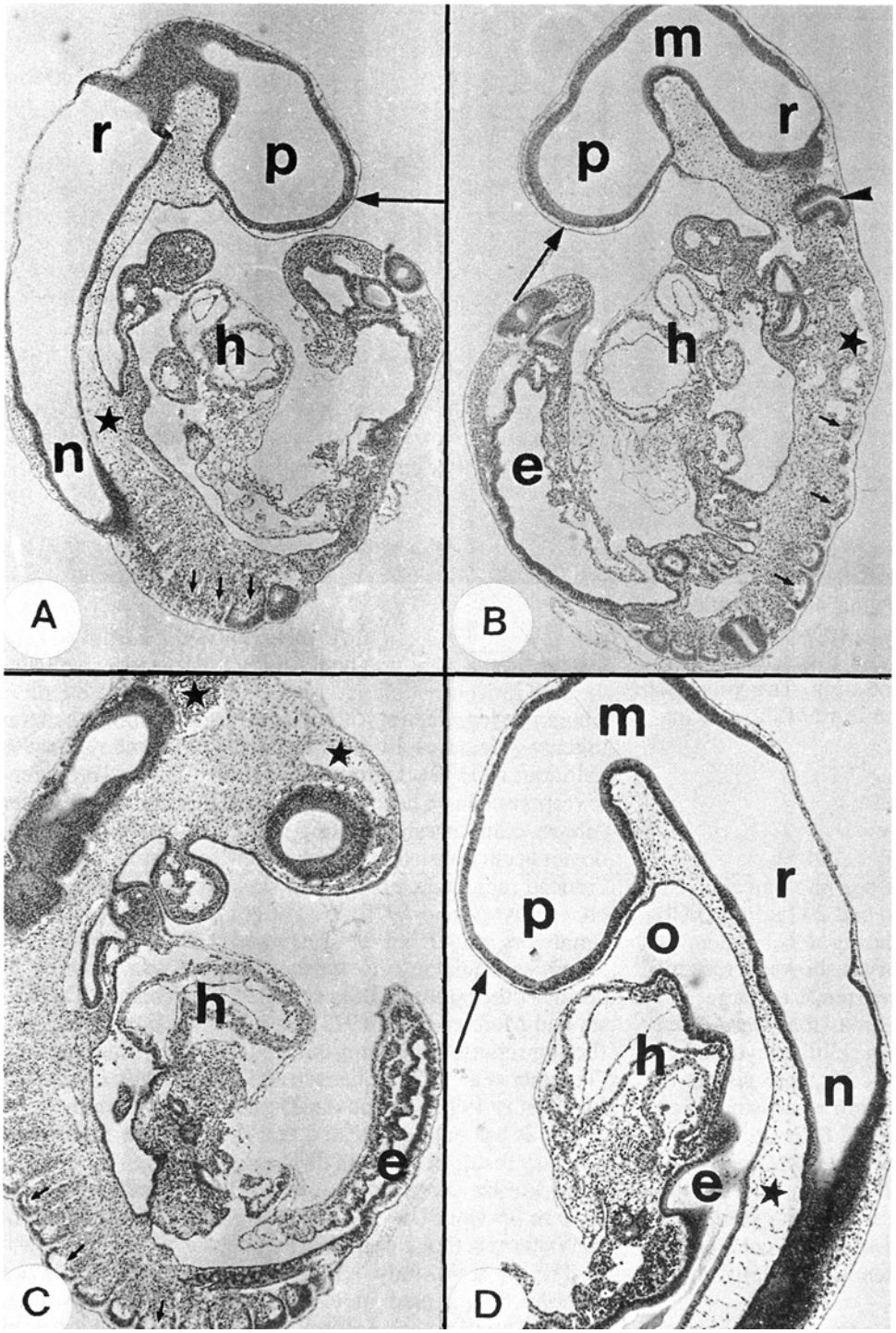

Fig. 3. Rat embryos; beginning of the culture period on day 9.5 of gestation, duration of the culture period 48 h. Sagittal section. **A** Treated with 150 µg/ml LiCl. No mesenchyme betweem neuroepithelium and ectoderm (*big arrow*). Less densely packed mesenchyme also in other areas (*). Somites (*small arrows*) of varying size. **B** Treated with 150 µg/ml LiCl plus 3.5 mM inositol. No mesenchyme between neuroepithelium and ectoderm (*big arrows*); somites (*small arrows*) of varying size. Less densely packed mesenchyme and formation of vessel-like cavities (*); irregular demarkation of ectodermal tube (*e*). **C** Treated with 150 µg/ml LiCl plus 14 mM inositol. Loosening of the mesenchyme and formation of large vessel-like cavities (*); somites (*small arrows*) of varying size; irregular contour of the entodermal tube (*e*). **D** Treated with 150 µg/ml LiCl plus 56 mM inositol. No mesenchyme between neuroepithelium and ectoderm (*big arrows*). Less densely packed mesenchyme (*). *p* = proencephalon; *m* = mesencephalon; *r* = rhombencephalon; *n* = neural tube; *h* = heart anlage; *e* = entodermal tube; *o* = primitive oral cavity

a developmental stage for lithium to induce open neural tubes. Furthermore, our historical data indicate that one open neural tube among 144 cultured embryos is a rate that falls within the range of spontaneously occurring neural tube defects in this system. If Hansen et al. had started culture on day 9, open neural tubes would probably have been detected in controls as well as in the lithium-exposed embryos. The incidence of open neural tubes in controls indicates, according to our experience, suboptimal culture conditions. Murine embryos exposed in whole-embryo culture exhibited an increased rate of open neural tubes, but only at the highest concentration (5 mEq lithium/l) tested (Hansen et al. 1990).

Lithium exposure of pregnant rats in vivo elicits cleft palate, eye and external ear defects, brain and digital defects and skeletal anomalies in the offspring (Wright et al. 1971; Marathe and Thomas 1986; Sharma and Rawat 1986). If cleft palate is excluded from a comparison of the in vivo and in vitro results because the in vitro system does not cover the period of palatal closure, then the comparative in vivo/in vitro results seem to be relatively well correlated.

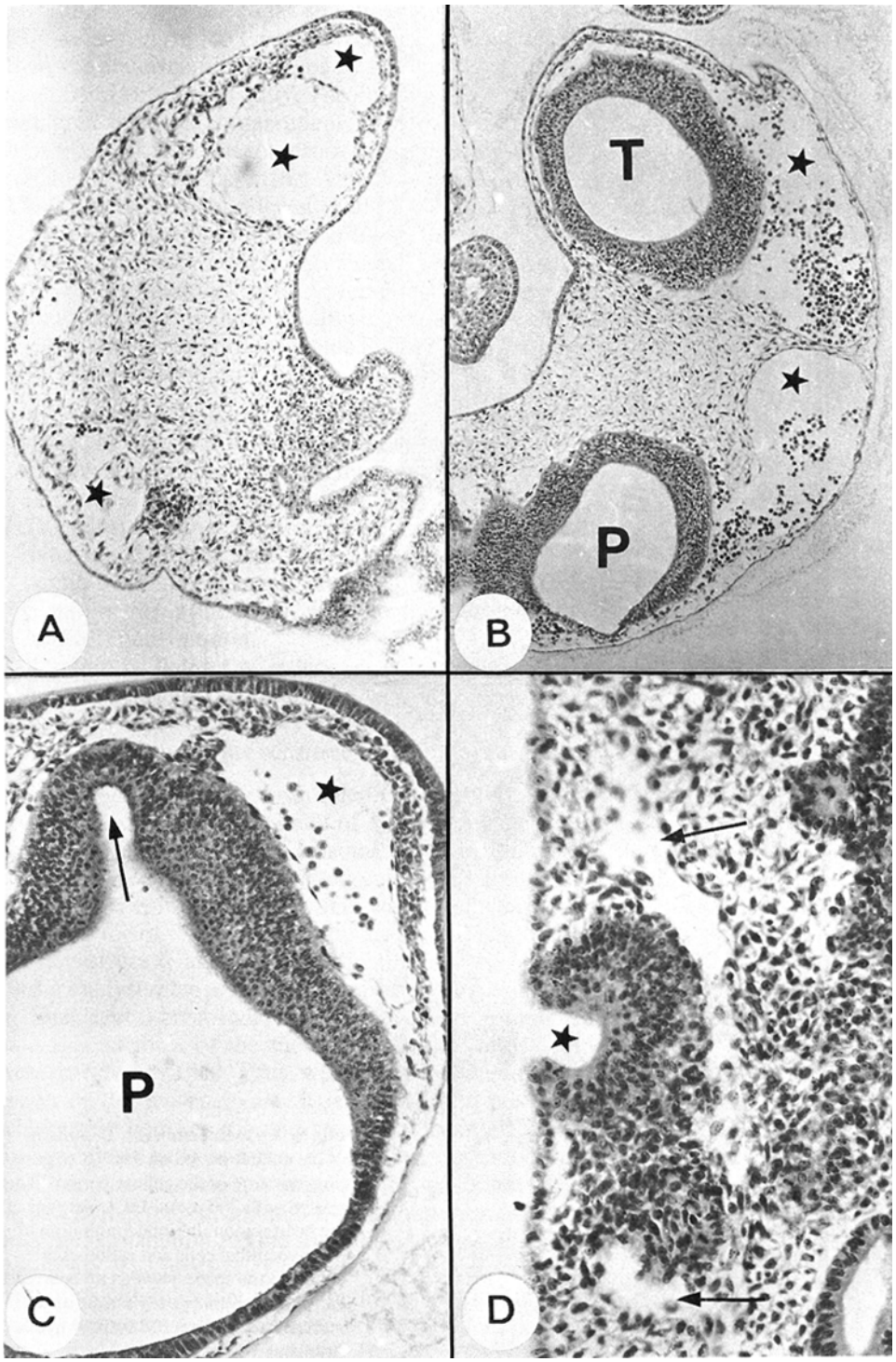

Fig. 4. Rat embryos; beginning of the culture period on day 9.5 of gestation, duration of the culture period 48 h. **A** Treated with 150 µg/ml LiCl. Lateral section. No optic process preceptible. Less densely packed mesenchyme and wide, vessel-like cavities (*). **B** Treated with 150 µg/ml LiCl. Less densely packed mesenchyme and wide, vessel-like cavities (*) in the head region that contain blood cells. **C** Treated with 150 µg/ml LiCL. Delayed formation of the optic process (*arrow*), wide-luminal vessels (*) in its vicinity. **D** Treated with 150 µg/ml LiCl. Otic vesicle (*) still open i. e. not yet sealed off. Less densely packed mesenchymal regions (*arrows*) in the vicinity. *T* = telencephalon; *P* = prosencephalon

We had hypothesized that lithium ions would induce open neural tubes in rat embryo culture. This hypothesis was based on the facts: firstly, lithium ions induce exencephaly when administered to mice in vivo. Although mice are generally more sensitive to the induction of exencephaly than rats in vivo, previous studies in our laboratory indicated that after valproic acid exposure rat embryos with exencephaly were resorbed. Thus, rat embryos exposed to valproic acid in vitro had open neural tubes, but rat embryos exposed in vivo did not display exencephaly because these embryos were resorbed (Klug et al. 1990). This result suggests that the response of rat and mouse embryos in whole-embryo culture to the induction of open neural tubes may be similar although different in vivo. Secondly, lithium inhibits hydrolysis of inositol monophosphates causing a depletion of inositol. Inositol depletion has been demonstrated to be a cause of open neural tubes in rat embryos in culture (Cockroft 1979, 1988). Thirdly, a previous study on rat whole-embryos grown in culture demonstrated that open neural tubes were induced by lithium

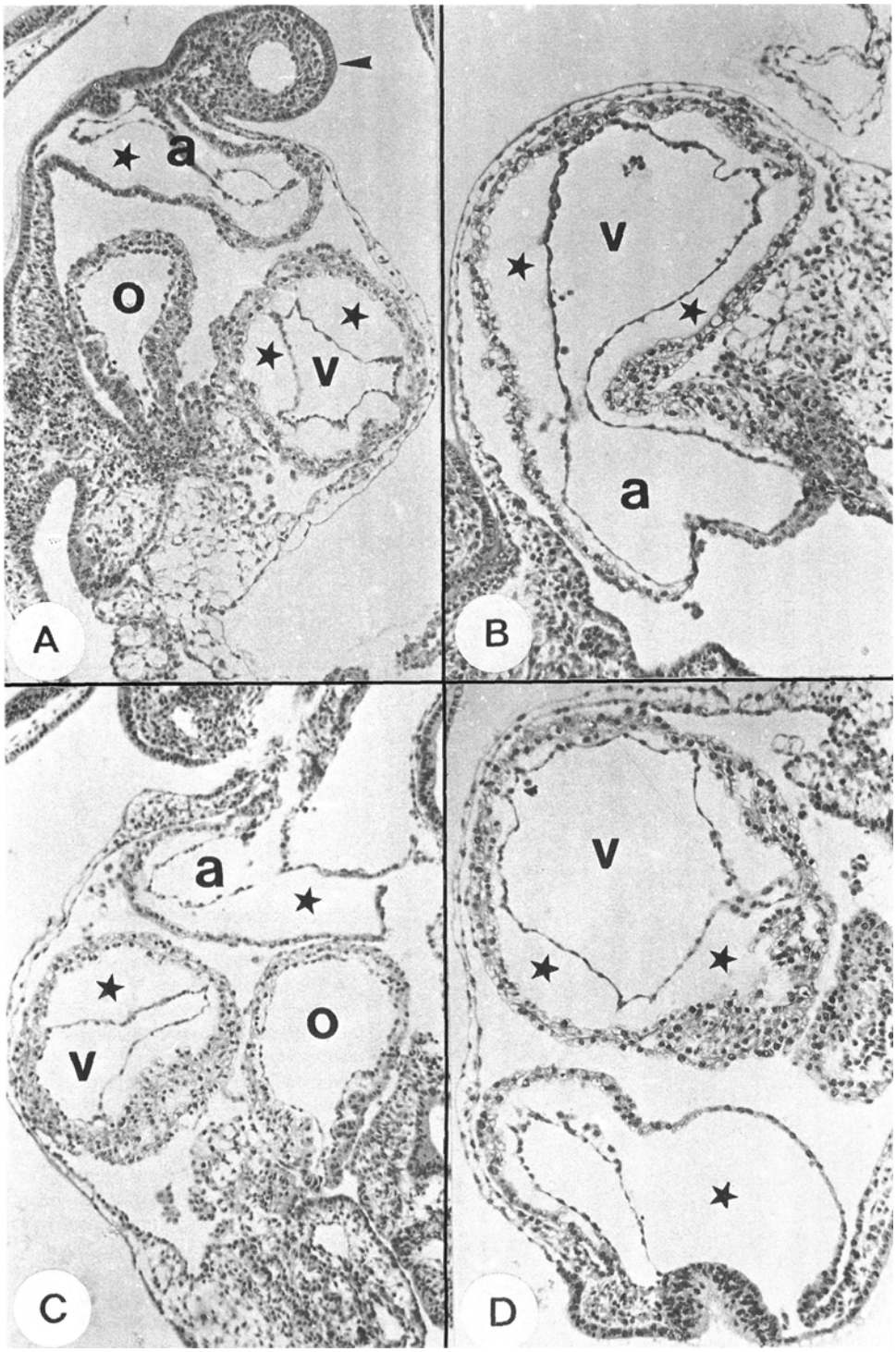

Fig. 5. A – D Rat embryos. Beginning of the culture period on day 9.5 of gestation, duration of the culture period 48 h, treated with 200 µg/ml LiCl. Sections of the heart region. Inhibited migration of the myoepithel cells and endothelial cells into the space between myocardium and endothelium (*). *a* = atrium anlage; *v* = ventricle anlage; *o* = outflow tract (truncus)

(Tesh 1988). However, a similar study had failed to induce open neural tubes in rat whole-embryos in vitro when culture was started at the ten somite stage of development (Hansen et al. 1990). In the present study, lithium ions also failed to induce open neural tubes in whole rat embryos grown in bovine serum.

It has been hypothesized that the developmental and neural actions of lithium are the result of inhibition of phosphoinositide metabolism (Berridge et al. 1989). Support for this hypothesis was reported in whole-embryo culture where lithium caused defects and altered the level of free *myo*-inositol and inositol-1-phosphate (Garcia-Palmer et al. 1988). More direct evidence was provided by the study of Busa and Gimlich (1989), in which they showed that either *myo*-inositol or the diacylglycerol analog phorbol myristateacetate were able to prevent lithium-induced teratogenesis.

The concentrations of LiCl found in the conceptus compartments after 48 h of exposure to a moderately embryotoxic dose (150 µg/ml) which induces abnormalities

(cf. Table 2), indicates that significant concentrations of LiCl are transferred to the embryo under these conditions. Thus, the abnormalities induced by LiCl do not necessarily result from yolk sac toxicity. The concentration of lithium measured in these compartments is compatible with the intracellular LiCl concentrations suggested to produce a maximum teratogenic effect which is in the range of 0.5–2.5 mM (Breckenridge et al. 1987).

In the present investigation utilizing the rat whole-embryo culture, *myo*-inositol was unable to antagonize the embryonic effects of LiCl. Several facts may serve as explanation for this and the following considerations must be taken into account if one wants to clarify the validity of the presented hypothesis:

1. The concentration of inositol in the culture medium might be too high, making it impossible to induce a relative inositol deficiency in the embryo by addition of LiCl to the culture medium.
2. The inositol concentration of the embryos should be determined before and after exposure to LiCl. This would be of importance in order to obtain direct knowledge on the influence of LiCl to the intraembryonic inositol concentration.
3. It would be helpful to induce an inositol deficiency in pregnant rats in vivo and then use these embryos for culture. Maybe only embryos growing under an absolute inositol deficiency are sensitive to the influence of LiCl.

Histological evaluation revealed that the mesenchyme was an important site of attack of LiCl. The head region appears to be affected first. In some embryos the mesenchyme between the neuroepithelium of the cerebral vesicles and the epithelium of the skin was missing. Since the mesenchyme of this region originates predominantly from the neural crest, a most interesting tissue was affected. Closer analysis also revealed loosening of the mesenchyme in other neural crest-independent areas. The somites and, as a special form of the mesenchyme, the cardiac jelly, were affected by LiCl. Thus, we are dealing with a general lesion of the mesenchyme. It is tempting to postulate a relationship between the migration activity of all types of mesenchymal cells and this effect. Since cytotoxic phenomena, i.e. necroses, could not be observed in the mesenchymal region, an inhibition of migration might be a possible explanation for the loosening or even absence of this tissue. The process of migration, however, comprises numerous individual steps, in which, for example, the contractile apparatus, the cell membrane dynamics and the extracellular matrix are involved. Hence, if the migration of the mesenchymal cells plays a role in this connection, its exact mechanism remains to be elucidated. In addition, it has not yet been clarified whether the discussed effect, which has been interpreted in terms of a migration inhibition, represents a direct or an indirect inhibition, e. g. due to a lesion of the yolk sac. However, under the indirect conditions more general lesions, e. g. necroses, would be expected to occur in other tissues as well.

Another finding might also be the result of the defects in the mesenchyme: development of often huge, sinus-like vessels in the loosened mesenchyme. At this developmental stage the wall of the blood vessles does not yet have a stable structure. It only consists of the endothelium. The vessels are stabilized by the surrounding mesenchyme. This capability is due to the water-binding of glycosaminoglycans and proteoglycans in the mesenchymal intercellular substance. That means a turgor tension exists in the extravascular space. Damage of the mesenchyme reduces this tension and, due to the blood pressure, the vessels dilate. A primary stimulation of the endothelial cells can, of course, not be excluded.

The situation in the heart is somewhat different. Here, in the beginning the matrix is formed by the endothelium and myoepithelium so that the turgor tension that stabilizes the endothelium is sufficient. Mesenchymal cells from the endothelium and myocardial cells from the myoepithelium (coelom epithelium) migrate into this pre-existing matrix. This type of cells can be demonstrated in control embryos as early as at the end of the culture period. In LiCl-treated embryos they were absent in large areas between endothelial tube and myoepithelial coat. An inhibition of migration may be the reason for this phenomenon.

The possible consequences of the absence of the mesenchyme cannot be judged with in vitro techniques. Due to the limited cultivation period it cannot be proven that the absence of the cells in the cardiac jelly or in the region surrounding the cerebral vesicles is of consequence for further development, but it is very likely that this is incompatible with further life.

However, it is also quite likely that migration and development of myocardium and mesenchyme can make up leeway. Findings showing a wrong position of the cardiac valves which develop rather early (Warkany 1988) might indicate a disturbance of the heart mesenchyme at this stage.

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