Mesodermal and Endodermal Differentiation of the Presumptive Ectoderm of *Triturus* Gastrula through Influence of Lithium Ion[1]

Since the finding of HERBST[2], LiCl has been proved to have the peculiar effect of modifying the embryonic development in some animals. In sea urchin embryos this chemical is known to act mainly on the animal half of the egg to cause transformation of the presumptive ectoderm into the endomesodermal tissues[3,4]. On the other hand, in amphibian embryos the effect of the chemical has been recognized to be the bringing-about of malformation of the nervous and sensory organs together with defect of the axial mesoderm[5]. Recently, however, the author[6,7] and ŌGI[8] have shown that the gastrular ectoderm of *Triturus pyrrhogaster*, treated with LiCl, undergoes not only neural but also mesodermal differentiations in response to those inductors which normally evoke only the neuralization of ectoderm, such as living prechordal plate, heat-killed organizer or guinea-pig liver. From this fact it can easily be assumed that in amphibian embryos, similarly as in sea urchins, the effect of Li ion is to affect the differentiation of presumptive ectoderm to give rise to meso- and endodermal differentiations. In order to get further evidences corroborating this view, the following experiments were undertaken.

Throughout the experiments, explantation was carried out with pieces of the presumptive ectoderm taken from the animal pole of early gastrulae of *Triturus pyrrhogaster*. In the first experiment, the ectoderm excised was immersed, before explantation, in 0.06 *M* LiCl solution of different pH value for 3.5 to 4.0 h. The pH was adjusted ranging from 7.0 to 9.8 by adding a small quantity of Li_2CO_3 to the solution. As control, the ectoderm was explanted after treatment with 0.06 *M* NaCl solution of the same range of pH value. In this case the adjustment of pH was made with Na_2CO_3. In the second experiment, the ectoderm was exposed to an ammonia solution of pH 11.8 to 12.0 for 2 to 6 min after treatment with Li-solution at pH 7.0, and then explanted. All the procedures were carried out at room temperature ranging from 23.5° to 26.5°C. Cultivation of the explants was done in Holtfreter's solution at 18°C for 2 to 5 weeks.

In the treatment with Na-solution disaggregation of the ectoderm always took place, whereas in the case of Li-treatment it occurred only when pH of the solution was above 9.2. But in either case reaggregation of the treated tissue was brought about by transferring the tissue into Holtfreter's solution. Generally the explants showed good differentiation, but quality of the tissues differentiated in the explants was different between Li- and Na-treatments as is seen in Table I. In the Li-treatments neural tissue was found and various kinds of mesodermal tissues (Figure 1, 2, 3, and 4) and sometimes endodermal tissue of gut-like appearance (Figure 5). On the other hand, in the treatments with Na-solution, neural tissue alone was found to be produced only when the pH value of the solution was above 9.2. Neither mesodermal nor endodermal differentiation was seen in this case. Further, it was noted that in the case of Li-treatments a tendency was found in which frequency and quality of the mesodermal tissues produced varied with change of the pH value of Li-solution. As seen in Table I, frequency of the mesodermal differentiation showed marked increase with rise of the pH, and at the same time notochord and somite became more frequently found than pronephros and blood cells. From this result, it seems likely that Li ion acts upon the presumptive ectoderm to cause meso- and endodermal differentiations, but the effect is modified by changing pH value of the medium.

On the other hand, double treatment of the ectoderm with Li-solution and ammonia gave a result comparable to that of the Li-treatment at higher pH values (Table II). Whereas the treatment with Li-solution alone brought about mesodermal differentiation only rarely, additional treatment with ammonia was followed by frequent production of not only notochord and somite but also endodermal tissue. Although it is a well-established fact that treatment of the ectoderm with alkaline solution brings about differentiation of the neural and mesenchymal tissues, no case has been found in which definitive mesodermal tissues are developed by the same treatment[9,10]. Therefore, we are inclined to consider that OH ion enhances the effect of Li ion upon the presumptive ectoderm of gastrula which elicits mesodermal and endodermal differentiations.

Tab. I. Differentiation of the explants treated with 0.06 *M* LiCl or NaCl at different pH.

pH	LiCl				NaCl			
	6.8 | 7.0	8.0 | 8.2	9.2 | 9.4	9.6 | 9.8	6.8 | 7.0	8.0 | 8.2	9.2 | 9.4	9.6 | 9.8
Available case	93	68	11	9	72	30	10	7
Neural tissue	–	–	5	7	–	–	4	4
Mesenchyme	9	37	2	–	–	–	–	–
Mesodermal tissue (total)	1 (1%)	16 (24%)	5 (45%)	7 (78%)	–	–	–	–
Blood	1	1	–	–	–	–	–	–
Pronephros	–	4	–	–	–	–	–	–
Somite	–	13	5	7	–	–	–	–
Notochord	–	–	5	6	–	–	–	–
Endodermal tissue (total)	29 (31%)	18 (26%)	3 (27%)	1 (11%)	–	–	–	–

Tab. II. Differentiation of the explants treated with ammonia solution after the treatment with 0.06 *M* LiCl of pH 7.0

	Ammonia-treatment alone	Li-treatment alone	Li- plus ammonia-treatments
Available case	37	93	30
Neural tissue	22	–	10
Mesenchyme	3	9	5
Mesodermal tissue (total)	–	1 (1%)	6 (20%)
Blood	–	1	–
Pronephros	–	–	–
Somite	–	–	6
Notochord	–	–	2
Endodermal tissue	–	29 (31%)	13 (43%)

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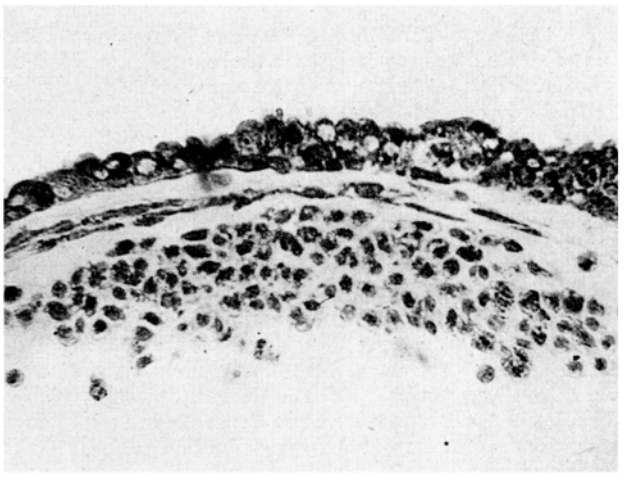

Fig. 1. Blood cells differentiated in Li-explant treated at pH 7.0 for 4 h.

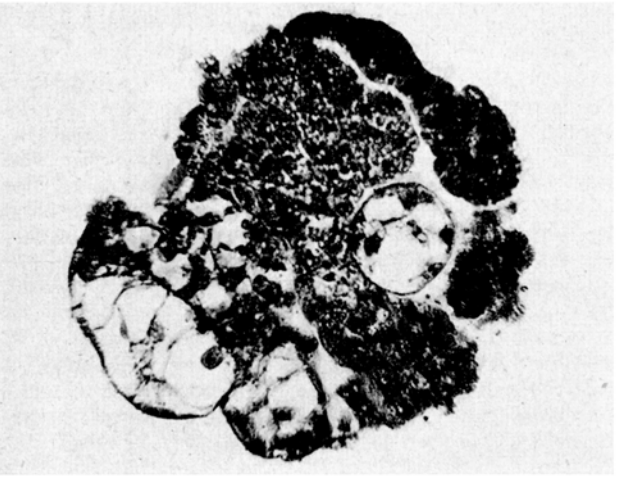

Fig. 4. Notochord and neural tissue differentiated in Li-explant treated at pH 9.8 for 3.5 h.

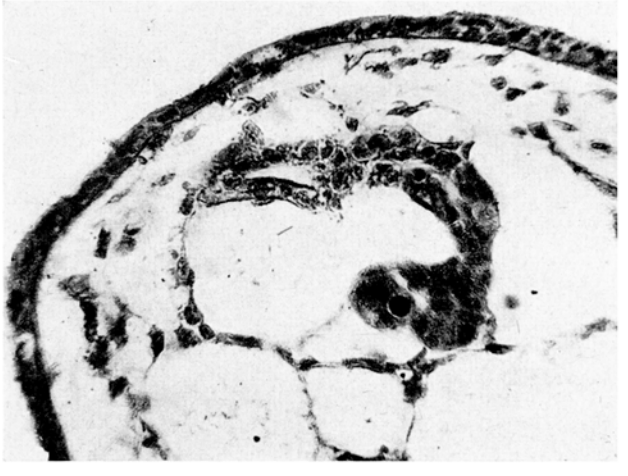

Fig. 2. Pronephric tubules differentiated in Li-explant treated at pH 8.2 for 4 h.

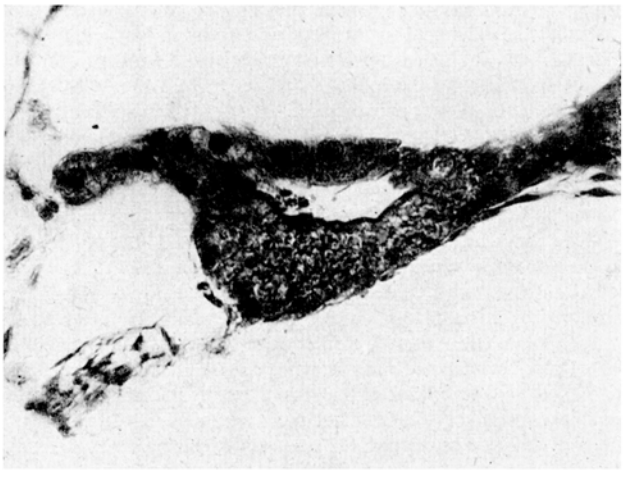

Fig. 5. Endodermal tissue of gut-like structure differentiated in Li-explant treated at pH 8.2 for 4 h.

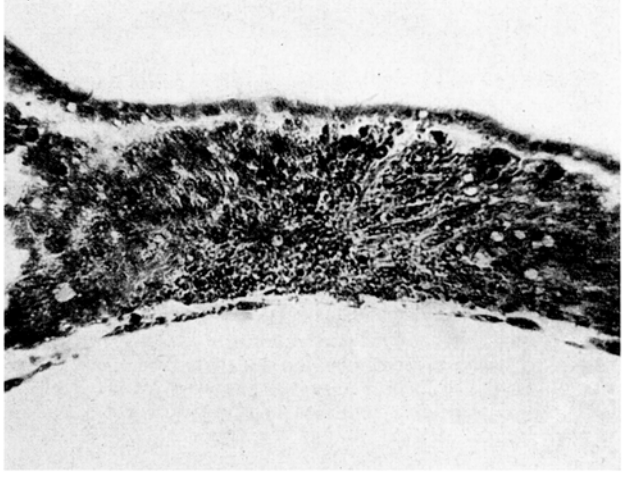

Fig. 3. Muscle differentiated in Li-explant treated at pH 8.2 for 4 h.

Zusammenfassung. Stücke des präsumptiven Ektoderms der jungen Gastrula von *Triturus pyrrhogaster* wurden nach 4stündiger Behandlung mit 0,06 *M* LiCl-Lösung explantiert. Das pH wurde durch Zusatz von Li_2CO_3 auf 7.0 bis 9.8 eingestellt. Die Explantate, die bei pH 9,2 oder 9,8 behandelt wurden, differenzierten sich in zunehmender Häufigkeit neben Neuralgewebe zu Chorda und Muskulatur. Bei Behandlung mit LiCl-Lösungen von pH 7,0 und 8,0 wurden nur Blutzellen, Vornierenkanälchen oder Muskulatur in geringem Prozentsatz produziert, jedoch weder Neuralgewebe noch Chorda. Diese Explantate ergaben aber Differenzierungen von Chorda und Neuralgewebe, wenn sie nach der Li-Behandlung kurz einer Ammoniak-Lösung von pH 12,0 ausgesetzt wurden.

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