Treatment with Lithium as a Tool for the Study of Animal-Vegetal Interactions in Sea Urchin Embryos

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Summary. The question about the nature of the effect of lithium on early sea urchin development is reexamined. Essential features of the morphogenetic changes of lithium treated embryos are followed with continuous comparisons with control embryos. The key to the lithium effect is the greater cytoplasmic susceptibility in the animal polar region as compared with the vegetal one. This is diagrammed in Fig. 23a–c on the basis of the double gradient concept. There is a decline of the animal gradient with increasing lithium concentration. As a consequence the level of differentiation of the terminal region becomes more and more vegetal, see \( an/veg \)-values in Fig. 23. The region suppressed by a prolonged exposure to lithium, e.g. 9–16 hours, cannot be restored. Nevertheless, there are data from previous research supporting the view that the primary effects of lithium are reversible within certain limits. However, when the normal balance is disturbed by decline of the animal gradient and particularly by suppression of its higher levels, there is a compensatory enhancement of the vegetal gradient system which stabilizes the suppression. As a consequence of the suppression of the higher animal levels, a certain accumulation of cells in an anterior direction has taken place in the blastula stage. The degree of accumulation reflects the degree of vegetalization. Later there is to varying extent a backflow of cells in the vegetal direction. It was shown how a great part of the blastula wall may have the aspect of an attachment zone (Fig. 7). The primary mesenchyme cells attach themselves only to a certain level of the ectoderm in which the relative \( an/veg \)-value is around 0.7 according to the conventions behind Fig. 23.

Sections of Carnoy fixed embryos were exposed to trypsin. It proved that the external cytoplasm of blastodermic cells in lithium treated embryos was more strongly attacked than the internal one. The latter showed a strong resistance to tryptic action. On the other hand, in the control embryos the inner part of blastodermic cells was completely digested with exception of the vegetal region including the attachment zone. The trypsin resistant structure may be preformed and may be responsible for the higher rigidity of the cytoplasm in lithium treated embryos (section IIIf).

It is proposed that in the period of lithium susceptibility, the colloidal state is most affected in the animal region, thereby creating a block to the diffusion of the animalizing substances which results in the shifts diagrammed in Fig. 23.

I. Introduction

Herbst (1892, 1893, 1896) introduced treatment with lithium added to sea water as tool in the experimental analysis of sea urchin development. The experiments of Herbst gave striking examples of how an external agent may transform regions of the embryo so that their differentiation may become different from what it should have been in normal development. The tool helped also to analyse interactions between different parts of the embryo, as for example, the role of the skeleton in the formation of the arms. Later the effect of lithium treatment has been tested on embryos in other animal groups as, for example, mollusces (Raven, 1952; Elbers, 1959) and amphibia (Masui, 1961; Ogi, 1961; Johnen, 1970).
Herbst stressed the effect of lithium on the vegetal part of the embryo manifested by an enlargement and evagination of the entoderm and tended to explain this as a growth phenomenon involving numerous cell divisions. Mac Arthur (1924) and Runnström (1928b) considered that the animal region of the embryo would be more sensitive to the action of lithium than the vegetal region. This conclusion was one of the starting points for the double gradient concept (Runnström, 1928a), according to which the proportion between animal and vegetal differentiation depend on two opposite intersecting gradients, one originating at the animal, the other at the vegetal pole.

It was further assumed (Runnström, 1933) that the gradients have chemical character. "Animalizing" and "vegetalizing" substances are diffusing from their respective polar centres. These notions became more concrete by the demonstration that animalizing and vegetalizing substances can be extracted from the eggs (Hörstadius et al., 1967). One essential trait in the concept of the double gradients is that these are in continuous competition during early development. If one of the gradients is impaired, the strength of the other one increases in a compensatory way. In lithium treated embryos, the compensation is inhibited by actinomycin D (Runnström and Markman, 1966; de Angelis and Runnström, 1969). This shows that transcription processes are involved in the compensation process.

Runnström (1928b) made some first experiments and observations which gave an indication of changes in colloidal state of the cytoplasm upon exposure to lithium. He showed, moreover (l.c.), that the lithium effect is counteracted by simultaneous addition of potassium or, at a lesser extent, of magnesium and calcium.

Ranzi and collaborators (for extensive review see Ranzi, 1962) carried out experiments on protein solutions extracted from animalized and vegetalized sea urchin or amphibian embryos. Among other results they reported that euglobin fraction from embryos pretreated with lithium are more resistant against urea and trypsin than are corresponding proteins from control embryos. Conversely, proteins from embryos animalized with NaSCN or o-iodoso-benzoic acid proved to be more sensitive to the same agent (Ranzi et al., 1957). By means of time laps cinematography Gustafson and Wolpert (1961) studied the morphogenetic cell movement in lithium treated embryos and found, for example, certain changes in adhesion properties of cells. Other aspects of animalization and vegetalization have been studied and reviewed by Lallier (1964).

The present paper aims at a more detailed study of the morphogenetic effect of lithium, particularly in order to test the validity of the double gradient concept. At the same time results were obtained which bear on the effect of lithium on the colloidal state of the cytoplasm. An attempt is made at coordination of morphogenetic and biochemical aspects of the lithium effect.

II. Material, Methods and Designations

The eggs and embryos of the sea urchin Paracentrotus lividus served as material. Washing of eggs, collecting of sperm and fertilization was carried out in usual way (see Runnström and Manelli, 1964). The eggs were usually transferred 20–70 min after fertilization to the lithium containing natural or artificial sea water, whereas parallel samples without