GENE DEPENDENCY OF VEGETALIZATION IN SEA URCHIN EMBRYOS TREATED WITH LITHIUM

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The polypeptide antibiotic actinomycin complexes with DNA in such manner that the DNA-dependent RNA-synthesis is suppressed. The replication of DNA, on the other hand, is not, or only slightly affected by actinomycin D (see Reich and Goldberg, 1964). This antibiotic has therefore become a widely used tool in experiments aiming at the analysis of the importance of the transfer of genetical information in synthetic processes with cells or cell components. Different steps of synthetic processes form the basis of embryonic differentiation. Their "gene immediacy" (Davidson *et al.*, 1963) may be analyzed by interrupting the gene transcription with actinomycin.

Actinomycin was used, for example, in studies on the sea urchin development by Gross and Cousineau (1963), by Lallier (1963) and by the present writers (Markman, 1963; Markman and Runnström, 1963), in studies on amphibian development and regeneration in *Acetabularia* by Brachet *et al.* (1963). For more recent work reference may be made to Denis (1964) and Zetsche (1964).

In the studies of Markman and Runnström, isolated animal halves of embryos of *Paracentrotus lividus* were exposed to rather low concentrations of actinomycin D (5 μg./ml.). In the animal halves kept as control in normal sea water an animalization occurred as usual (see Hörstadius, 1935). This means an increased extension of the acron region, consisting of cylindrical cells carrying the stereocilia (about 70 μ long), forming the so-called ciliary tuft. Its extension gives a certain semi-quantitative measure of animalization. This depends on a rupture of the normal balance between the intercepting animal and vegetal gradients, the presence of which is supported by numerous experiments (Hörstadius, 1935, 1949; Runnström, 1933, 1954, 1964). Conversely, in the animal halves exposed to actinomycin, the animalization was much less pronounced, the acron and ciliary tuft hardly extending beyond the confines found in whole normal embryos. A certain although very weak tendency to formation of mesenchyme cells prevailed at the vegetal pole of the isolated animal half. The interpretation must be that transfer of genetical information is involved in the animalization of the animal half. Owing to the existence of the animal and vegetal gradients, a continuous system of different levels is formed. Gradually, an interaction between the cytoplasm and the nuclei intensifies which leads to a selective induction of gene activity. According to our view, the selection depends on the level at which the single nuclei are located in the double gradient system. The induction of gene activity leads to a certain unification of the levels to different discontinuous zones (Runnström and Immers, 1966). This process is blocked if the DNA-dependent formation of RNA is suppressed by actinomycin.

The weakening of the animal differentiation in the experiment referred to should thus depend on the block exerted by actinomycin against the production of messenger RNA, serving as template for proteins characteristic of the most animal zone of the embryo. With a different procedure Lallier (1963) demonstrated also the gene dependency of the animal differentiations.

In the present paper the question is raised as to whether transfer of genetical information is a prerequisite also for vegetalization. This has been brought about by the classical method of Herbst (1892) : exposure of the embryos to sea water to which LiCl has been added. It will be shown in the following that actinomycin counteracts the vegetalizing effect of lithium. There is an apparent divergence between this result and those briefly presented by Lallier (1963).

Material and Methods

The material used was *Paracentrotus* (*Pa.*) *lividus* from the Gulf of Naples. The methods of removing the gametes, washing the eggs, etc. were the same as previously used by these writers. The lithium was of the grade *pro analysi.* The actinomycin D was a gift from the research laboratories of Sharp and Dohme. In each experiment eggs from one female, fertilized with sperm from one male, were used. The volume of the test samples was 6 ml. with the same number of eggs in each of them (estimated to about 2000 eggs per ml.). After a certain time of exposure to lithium, actinomycin, or mixtures of both, as much as possible of the supernatant fluid was removed from the sedimented embryo by means of a pipette connected with a water pump; pure sea water was added to give a volume of 18 ml. After sedimentation of the eggs the same procedure was repeated. In this way the concentrations of the tested substances were reduced in the culture medium to less than 1% of the concentration prevailing during the time of exposure. The concentration after washing was more than 10 times below the limit at which any action could be traced.

Survey of the Main Results

Preliminary experiments were carried out with lithium concentrations varying between 0.033 and 0.049 *M* lithium; the concentrations of actinomycin D were 12.5 or 16.5 μg./ml. In the experiment pertaining to this report the exposure occurred during the cleavage and blastula stage (3–8 or 9 hours after fertilization). Variations in the degree of response depended as usual not only on the mode of exposure but also on the different susceptibility of materials from different batches of eggs.

It seemed suitable to use a lithium concentration which gives a clearly distinguishable but limited vegetalization. This should reduce the after-effect of pooled lithium on the embryos after return to normal medium. In the final procedure the eggs were kept for three hours after fertilization in pure sea water. The egg suspension was then diluted so as to obtain the following for test samples:

C_o Egg suspension in sea water
C_{act} Egg suspension in sea water with 12.5 μg./ml. actinomycin D
Li_o Egg suspension in 0.033 *M* lithium
Li_{act} Egg suspension in 0.033 *M* lithium with 12.5 μg./ml. actinomycin D

In Table I, one particular experiment with *Pa. lividus* (N 65, exp. 77) is recorded in a more detailed way. The exposure lasted for 5.5 hours (from 3 to 8.5 hours after fertilization). At different intervals embryos from the four test samples were fixed in neutral 4% formaldehyde and subjected to a detailed examination. A survey of certain features of the embryos is given in Table I under A-D. Reference

TABLE I, A–D

Survey of features of embryos of Pa. lividus treated according to scheme given in the text

A. Embryos fixed 22 hours after fertilization.

C_O Bilateral gastrulae (prisma stage).

C_{act} Early stage of gastrulation. Primary and secondary mesenchyme cells in vicinity of the archenteron.

Li_O Archenteron invaginated. Bilateral symmetry of the ectoderm is indicated. Primary mesenchyme cells arranged in a ring, the position of which was somewhat more animal than in C_O.

Li_{act} No, or slight invagination of archenteron. Bilateral symmetry of the ectoderm only slightly indicated. Smaller primary (diameter *ca. 5* μ) and larger secondary mesenchyme cells arranged in two ventro-lateral groups.

B. Embryos fixed 27 hours after fertilization.

C_O Early plutei; pigment cells have appeared.

C_{act} Gastrulae at the same level of differentiation as in *A* C_O, but bilateral symmetry more pronounced, and acron more extended than in *A* C_O. Acron participates in the formation of the ciliary band. Tendency to accumulation of primary in ventral, and of secondary mesenchyme cells in dorsal direction (*cf.* Markman, 1963, Fig. 1). No pigment cells.

Li_O Vegetalization clearly indicated. The two attachment zones of primary mesenchyme were displaced in varying degree in the animal direction, their position often asymmetrical. Acron weakly differentiated. In 40–50% of the embryos the archenteron, in particular its animal region, was enlarged (Fig. 2). The archenteron could be completely invaginated but the invagination often was incomplete, forcing the anal ectoderm also to protrude (Fig. 3). Beside a main archenteron of normal size, accessory small archentera were sometimes present (Figs. 4 and 5).

Li_{act} Gastrulae. In contradistinction to Li_O, archenteron of normal proportions. A tendency prevailed to assemble the primary mesenchyme cells in ventral and the secondary ones in dorsal direction (*cf.* C_{act}). No, or few pigment cells.

C. Embryos fixed 53 hours after fertilization.

C_O Plutei with four arms. Ciliary tuft replaced by motile cilia. Stomodeum united with oesophagus. Pigmentation normal. Beginning involution. Distance end of apex to top of anal arms: 230 $\pm$ 13 μ (n = 10).

C_{act} Embryos without arms, acron relatively larger than in C_O. Ciliary tuft of normal or slightly extended type still present. Relative to C_O, the intestine was under-dimensioned and incompletely subdivided into compartments (Figs. 6 and 7). Bilateral skeleton rudimentary. Number of pigment cells has increased. Apical region short. Animal-vegetal distance 164 $\pm$ 2.6 μ, (n = 12).

Li_O Plutei; a high percentage of the specimens with a varying degree of slight vegetalization, as specified under *D.*

Li_{act} Embryos with no, or rudimentary arms, no vegetalization. The outer profile different from C_{act}, in particular by the formation of a proportionate concave oral field (Fig. 8), sometimes with a stomodeum. The intestine subdivided into its three compartments. Ciliary tuft present in a number of the embryos (Fig. 8), but may be absent. Skeleton better developed than in C_{act}; oral and anal rods rudimentary.

TABLE I—(*Continued*)

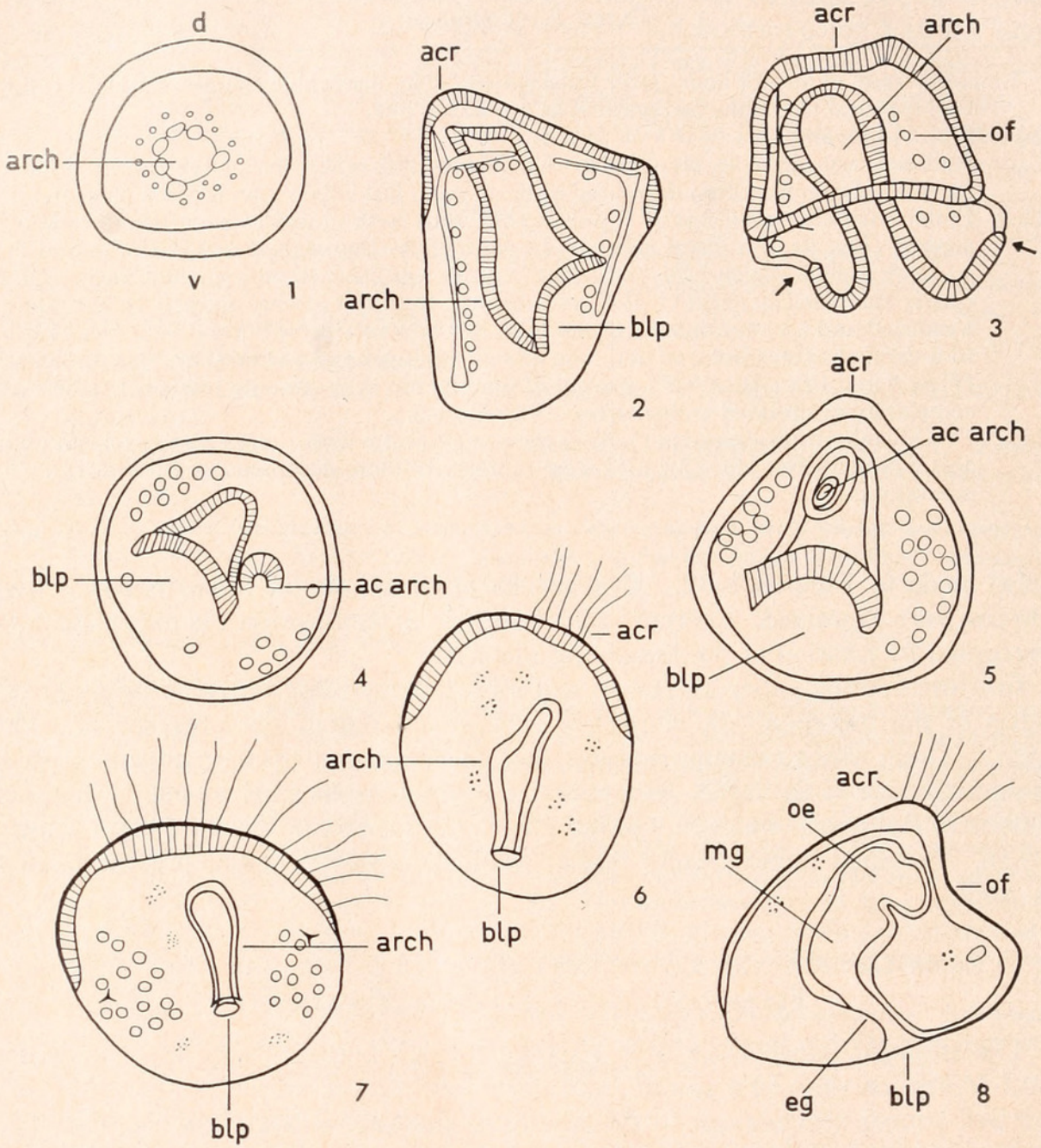
D.	Embryos fixed 70 and 79 hours after fertilization. No observable changes during this interval; material may thus be regarded as a uniform one.
C_0	Plutei in involution.
C_{act}	Rudimentary plutei in involution. No further differentiation of the intestine has occurred. Acron has often decreased after stage *C*, but ciliary tuft is often present.
Li_0	Plutei with elongated apical region; apical rods with club-like ends; these rods often crossed (Fig. 9) in a distal region. In contrast to the vegetal region of the oral field, its more animal region (the "oral lobe") often was reduced and asymmetrical. Of 166 closely examined larvae: (1), 36% were normal, or almost normal plutei; (2), 20% showed a reduced and (or) asymmetrical oral lobe, but archenteron of normal size; (3), 34% had to a varying extent reduced oral lobe and an enlarged and partially evaginated intestine (Figs. 9 and 10). In <0.5% typical lithium larvae with strongly reduced ectoderm and completely evaginated archenteron. Pigmentation.
Li_{act}	Of 235 embryos examined: (1), 88% were small plutei with rudimentary arms and blunt apical region (Fig. 11); (2), 12% were plutei with more developed arms (Fig. 12). Pigmentation.

is also made to Figures 1–12. Besides the observations on fixed material, living embryos were examined. Seventy-nine hours after fertilization the experiment was interrupted by fixation of the remaining material.

In a forthcoming paper Markman will report about quantitative autoradiographic studies of the incorporation of nucleic acid precursors in the presence of actinomycin. These measurements indicate that the exposure to actinomycin caused a strong inhibition of the RNA synthesis also in the present experiments. In experiments with *Psammechinus* (*Ps.*) *miliaris* from the vicinity of Kristineberg Zoological Station, Markman found results similar to those obtained in the present work on *Pa. lividus.*

SOME DETAILS AND COMMENTS CONCERNING THE MORPHOGENESIS FOLLOWING WEAK LITHIUM TREATMENT

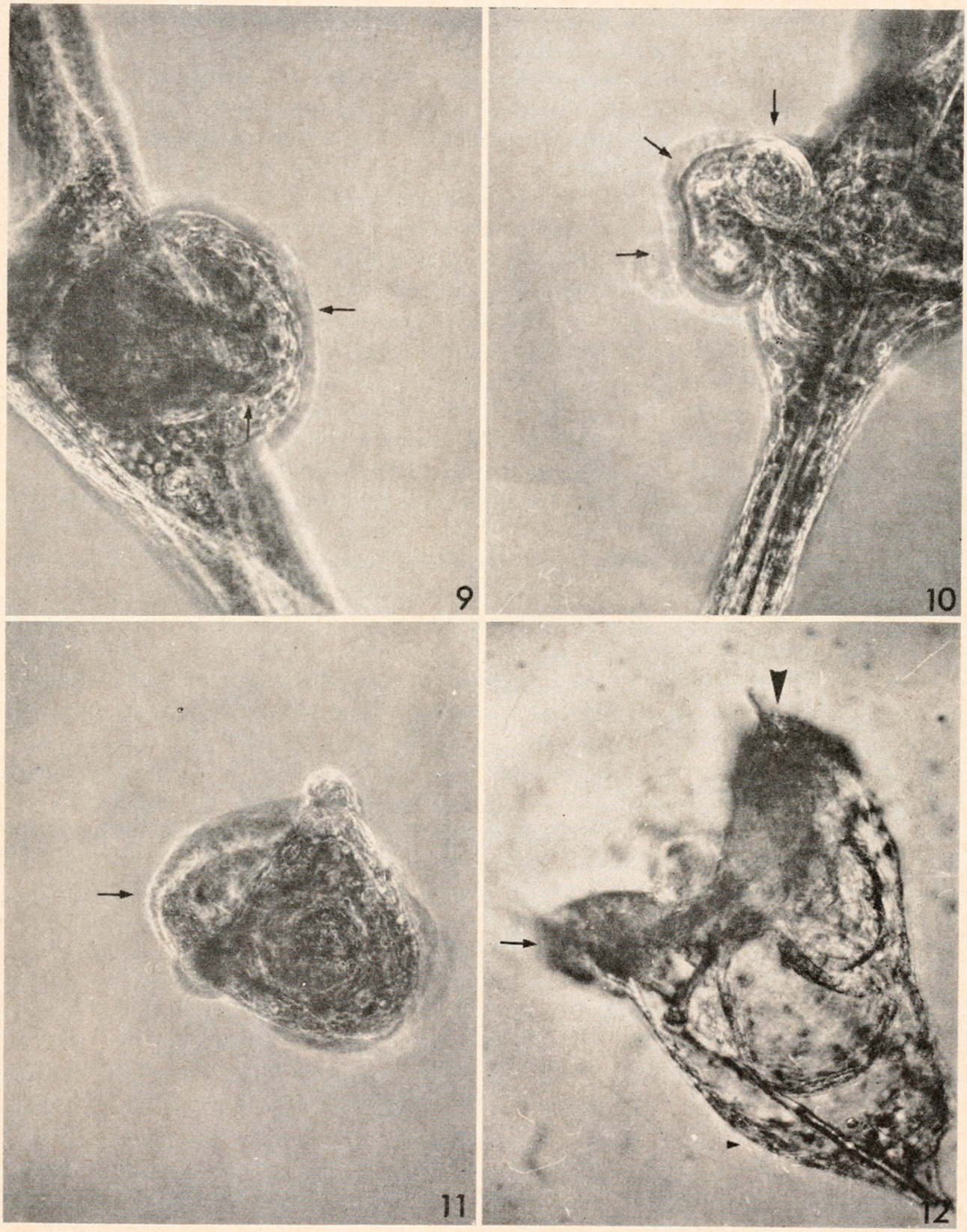
The embryos which have been exposed for 5.5 hours to sea water containing 0.033 *M* lithium showed a gamut from normal or almost normal plutei, plutei with defective animal oral regions to those with moderately enlarged archenterons. At the concentration of lithium and time of exposure used, only very few (<0.5%) were typical lithium larvae with completely evaginated archenteron and strongly reduced ectoderm (Table I D, Li_0). The moderate enlargement of the archenteron was confined to its animal posterior region, as shown in Figures 2 and 3 (Table I B, Li_0). Of particular interest is the formation of smaller accessory invaginations (Figs. 4 and 5). In the present experiment the zone derived from the border regions between veg_1 and veg_2 (see Hörstadius, 1935) often seems to fluctuate with respect to its state of differentiation. Either the animal or the vegetal pathways dominate at about the same animal-vegetal level. Similar fluctuating states were observed by Lindahl (1936) in embryos from eggs which were pretreated with thiocyanate in calcium-free medium, fertilized in normal medium and thereafter transferred to sea water containing lithium. According to Hörstadius (1936), the fluctuations are also found when isolated animal halves are subjected to a somewhat delayed treatment with lithium.



Designation in FIGURES 1–8: *ac arch*, accessory archenteron; *acr*, acron; *arch*, archenteron; *blp*, blastopore; *eg*, endgut; *mg*, midgut; *oe*, oesophagus; *or*, oral field. Reference is made to Figures 1–8 in Table in the following places: *A*, Li_{act} (Fig. 1); *B*, Li_0 (Figs. 2–5); *C*, C_{act} (Figs. 6–7); *C*, Li_{act} (Fig. 8) Ca. 250 ×.

The additional archenterons were found also in the pluteus stage. They were never subdivided in more than two compartments, but more than one additional archenteron was sometimes observed. In one embryo of 79 hours, one additional intestine had two compartments, but another was not subdivided. Moreover, the midgut of the main intestine showed two buds, one in ventral, another in dorsal direction, indicating a fluctuating state even at a more vegetal level.

Figure 3 represents an embryo 27 hours old (Table I B, Li_0), in which the archenteron showed an obvious enlargement. Its most animal part had not invaginated but formed a field of cylindrical cells (delimited in Figure 3 by arrows).



Designations in FIGURES 9–12: Figure 9, *horizontal arrow:* ectodermic bulge with squamous epithelium; *vertical arrow;* enlarged intestine opening through the bulge; 320×. Figure 10, *vertical and horizontal arrow:* bulge; oblique arrow, opening of the intestine. 160×. See for Figures 9 and 10, Table 1; *D*, Li_0. Figure 11, *horizontal arrow:* oral field with stomodeum. 160×. Figure 12, *vertical arrow head:* oral lobe with rudiment of oral arms with projecting skeleton rods; *horizontal arrow:* anal arm with skeleton rod; *lower arrow head (small):* apical region with rods; 320×. See for Figures 11 and 12, Table I, *D*, $Li_{act.}$

This field surrounded the entrance into the invaginated more vegetal part of the archenteron. The field showed a sharp delimitation against a lower epithelium belonging to the apical ectoderm, whereas the oral ectoderm formed a typical oral field, surrounded by a ciliary band; no stomodeum had appeared, and a skeleton had been formed only at one side of the embryo. In further development, embryos similar to that represented in Figure 3 gave rise to the type of plutei represented in Figures 9 and 10. A bulge was formed at the anal side of the embryo. The hind gut penetrated through this bulge and opened to the exterior. In the embryo of Figure 9, the opening was surrounded by a rim of entoderm corresponding to a non-invaginated part of the archenteron; the greatest part of the bulge consisted, however, of squamous epithelium of apical (dorsal) character. This ectodermic region was thus considerably enlarged at the cost of more animal ectoderm. In particular, the oral lobe was strongly reduced.

In the embryo represented in Figure 10 the lobe (arrows) had a more cylindrical character. It was perforated by the intestine which opened to the exterior by a funnel-shaped region. In a further example, two ectodermic bulges were found. One of these had squamous epithelium; the intestine had its opening in the wall of this bulge. The other one consisted of a rather high cylindrical epithelium; the communication between the interior of this bulge and the general blastocoel was rather narrow. The oral lobe was almost suppressed in this embryo, which exemplifies how presence of oral lobe and that of ectodermic bulge tends to exclude each other.

The cylindrical character of the epithelium seems to indicate that the bulge has a more animal character than that corresponding to its level in the animal-vegetal gradient system. The fluctuating state in the border zone may thus involve both ectoderm and entoderm. Its cause must be a weakening of the control, normally exerted by the animal polar region. In the present experiment, this control was impaired by the exposure to lithium in a concentration which did not bring about a full predominance of the vegetal pathways. Under these circumstances a labile state is produced so that the differentiation may easily be switched in one direction or the other. This indicates that rather sharp threshold effects prevail in the differentiation along the animal-vegetal gradient system.

The data which have been discussed in the previous paragraphs recall the "anarchy of gradients," described by Runnström (1933). Under the present conditions, addition of actinomycin D (Table I, Li_{act}) prevents this anarchy. In one case of about 300 a bulge appeared in the test Li_{act}. This bulge was of ectodermic character and exerted an evident action on the secondary mesenchyme cells (see Runnström and Immers, 1966).

Considerations of the Gene Dependency of the Lithium Effect

Table I, Li_0 and the preceding section made clear that lithium in the concentration and time of exposure used (0.033 *M* in sea water for 5.5 hours) was able to bring about a distinct vegetalization. If, in addition, the test medium contained 12.5 μg./ml. actinomycin D, plutei arose which usually deviated from the normal by being smaller or by having somewhat shorter skeleton rods and arms than normal plueti. This obvious approach to normal development demonstrates that gene

activation and transfer of genetical information is a necessary link in the vegetalizing action of lithium. A weak effect of lithium reveals itself as a defective differentiation of the oral lobe (Runnström, 1928; Mastrangelo, 1965). This indicates that lithium affects primarily the animal anabolic pathways. Thus the synthesis of the specifically animal proteins or protein complexes may be impaired during the lithium-sensitive period, which, according to Lindahl (1940) and Bäckström and Gustafson (1953), is about 3–9 hours after fertilization.

Actinomycin D was found to remove the fluctuating state discussed in the previous section. Gene activity is thus involved in the switches occurring in the border zone between ectoderm and entoderm.

Runnström (1928) showed that lithium has a condensing action both on egg surface and interior of the eggs. Ranzi and collaborators (for comprehensive review, see Ranzi, 1962) showed *i.a.* that the proteins of embryos vegetalized by treatment with lithium were less easily attacked by trypsin than the proteins of control embryos.

Centrifugation experiments showed that lithium delayed the stratification of cytoplasmic particles relative to the stratification obtained in control eggs (Ranzi, 1962, p. 232; Lallier, 1955).

It seems possible that lithium has an effect on intra- and intermolecular bonding in certain cytoplasmic proteins which increases their tendency to aggregation. This view was substantiated in model experiments by Ranzi and collaborators (see Ranzi, 1962, *e.g.*, p. 237). The effect of lithium involves a competition with potassium (Runnström, 1928; Lindahl, 1936).

Molinaro and Hultin (1965) found a high potassium optimum for the incorporation of amino acids in cell-free systems from *Pa. lividus*. When lithium substitutes for potassium the amino acid incorporation is very low. Mastrangelo (1965) found, however, no effect on the amino acid incorporation in whole embryos subjected to vegetalizing concentrations of lithium.

In the living embryo lithium must, in the concentrations used, interfere with some weak link in the chains of physical and chemical events involved in the differentiation of the embryo. The constriction experiments of Hörstadius (1938) indicate the great importance of diffusion processes in this connection (for discussion, see Immers and Runnström, 1965). The primary lithium effect may particularly impede the diffusion of the animalizing agents which may undergo an aggregation or (and) become bonded with other components of the cells. The specific inducing effect of the animalizing agents on gene transcription is thus inhibited and, as a consequence, the animal anabolic pathways are suppressed to varying extent.

Conversely, the vegetalizing agents would be more independent of, or more protected against the cytoplasmic changes caused by lithium. An unbalance in the animal-vegetal gradient system would thus arise. This unbalance is enhanced by transient exposure to anaerobic conditions or to uncoupling agents (for survey, see Runnström, 1964).

Structural changes in the nuclei have also been described as an effect of exposure to lithium (Runnström, 1928; Hagström, 1963). It seems, however, less probable that lithium could exert its effect directly on the gene transcription with the remarkable degree of specificity observed.

Figure 13 refers again to the elaborate experiment of Table I. *An* and *Veg* indicate here the centers of production of the animalizing and vegetalizing agents, and the wedges their concentration gradients towards the opposite poles. These agents, or at least their precursors, are certainly present before the fertilization, as indicated also by the data presented by Giudice and Hörstadius (1965). A later replenishment mediated by genes is, however, probable. Figure 13 shows also how the agents may have a different influence on equal genoms located at two different levels in the double gradient system. The ratio of concentrations of animalizing and vegetalizing agents at the two levels is different and this is supposed to cause the activation or repression of different genes or operons (Jacob and Monod, 1963).

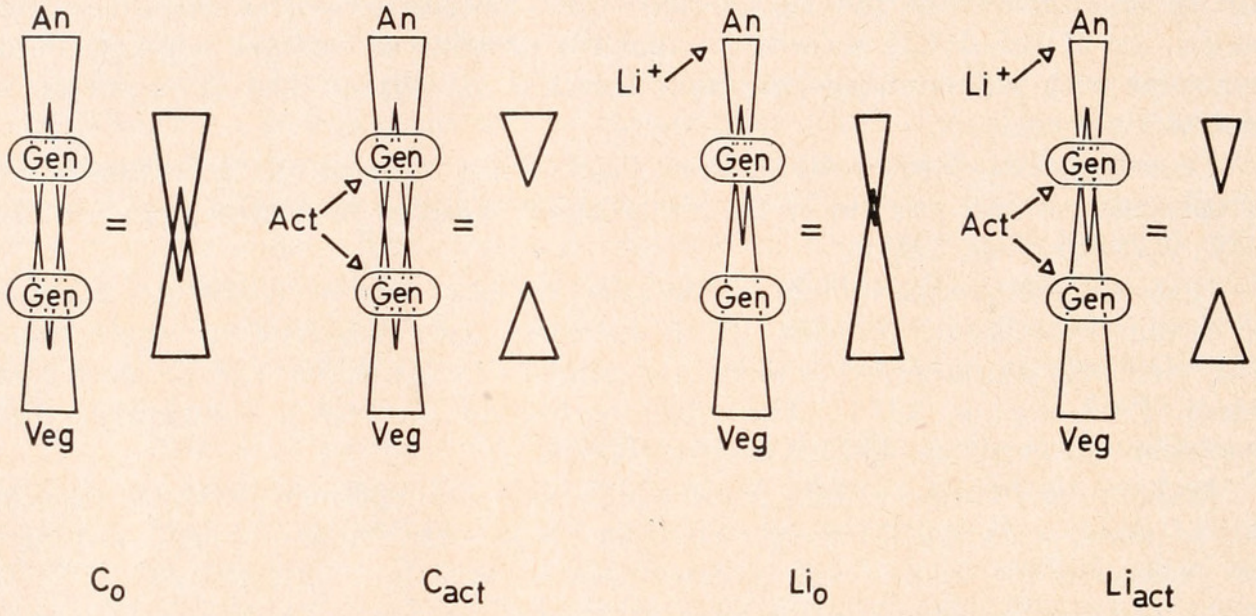


FIGURE 13. Diagrammatic representation of the state of chemo-differentiation in embryos exposed to sea water (C_o), to sea water with actinomysin D (C_{act}), to sea water with lithium (Li_o) and to sea water with lithium and actinomycin (Li_{act}). Each part of the diagram referring to one test (C_o, etc.) is divided into two parallel diagrams. The one to the left represents the gradients of animalizing and vegetalizing agents and their action on equal genoms at different levels during the exposure to the added substances. Actinomycin D blocks the transcription of information from the genom and removes thus, at least partially, the effect of the animalizing and vegetalizing agents. To the right, the extents of the animal and vegetal pathways are indicated in the embryos after transfer to normal medium. See, further, text.

To the right in each of the schemes C_0-Li_{act} in Figure 13, the results of the assumed interactions are symbolized. The wedges indicate here the relation between animal and vegetal anabolic pathways shortly after transfer to normal medium. C_0 shows the normal conditions in which the inducing animalizing and vegetalizing agents are in balance. In C_{act}, actinomycin D blocks the transfer of information both for the animal and the vegetal anabolic pathways. The consequence is a considerable inhibition of the differentiation of the whole embryo. When regulation processes begin after transfer to normal medium, the chances are about equal for the animal and the vegetal pathways, and an approach to a normal balance between these will occur.

In Li_0 the effect of the lithium treatment should be a decrease in the activity

of the animalizing agents which induce the transfer of information for the animal anabolic pathways.

In Li_{act} as in C_{act}, actinomycin D blocks the transfer of information both for the animal and vegetal anabolic pathways. In this way, the inducing action of the vegetalizing agent is also excluded and the formation of additional entoderm which occurs in Li_0 is suppressed. This is the main piece of evidence to show that the vegetalization brought about by lithium is dependent on transfer of genetic information. One must presume that under the effect of lithium the action of vegetalizing agents is relatively increased even in the most animal region of the embryo, because the competition with animalizing agents is decreased. The vegetalizing agents reinforce then the vegetal anabolic pathways in the animal region. The presence of actinomycin D will, however, quench this effect of lithium and contribute to making the embryos subjected to the double treatment more balanced than those subjected to lithium alone.

In reality, C_{act} and Li_{act} are not as similar as should be expected from the outlined scheme. In the former, the archenteron was somewhat underdeveloped and poorly differentiated, even if a certain subdivision into compartments occurred. In still earlier stages the archenteron seemed condensed in an irregular way and had often no visible lumen. This may be due to the quenching effect of actinomycin D on the transfer of information necessary for certain secretions or components of the cell surface which are important for the capacity of the cells to slide against each other during mesenchyme formation and invagination of the archenteron. The impairment of the cell movements may have secondary effects on the functional, as well as on the morphological state of the archenteron. The moderating effects on acron and ciliary tuft may be reduced. The state of the mesenchyme and archenteron in C_{act} is to some extent reminiscent of that found in embryos which are raised in sulfate-free sea water (Runnström *et al.*, 1964; Immers and Runnström, 1965).

In Li_{act} the archenteron had more normal proportions than in C_{act}. The effect of lithium may not be completely eliminated following the combined treatment. A weak lithium effect may still be capable to counteract the tendency to animalization, prevailing in C_{act}. The consequence will be that the embryos, after transfer to normal medium, undergo a development in which the animal-vegetal balance is normal. The data of Giudice and Hörstadius (1965) exclude rather effectively that actinomycin D could combine directly with the vegetalizing agents and thus eliminate their action.

The discussion in this section may be reassumed by the following tentative conclusions. Lithium changes primarily the state of certain cytoplasmic proteins. The animalizing agents which induce transfer of information for the animal anabolic pathways are particularly sensitive to this primary lithium effect. This leads to a disturbance of the animal-vegetal balance which promotes the action of the vegetalizing agents. As a consequence the vegetal anabolic pathways are reinforced. This reinforcement is quenched by actinomycin D, which shows that gene activation and transfer of genetic information are involved in the vegetalizing action of lithium.

Lallier (1963) has presented data which seem to be at variance with those reported here. Thirty minutes after fertilization eggs of *Pa. lividus* were brought into sea water with 1.3×10^{-2} *M* lithium and 10 μg./ml. actinomycin D. The exposure lasted for 20 hours and thereafter the developing embryos were brought

into normal sea water. Under these circumstances a stronger vegetalization was found after combined treatment than after treatment with lithium alone. Results of the kind obtained by Lallier may have the following explanation. Following return to normal sea water after a prolonged exposure to lithium in sea water, a certain pool of lithium may remain within the embryos which counteracts a regulation in direction of normal balance. If the embryos have been exposed to a prolonged treatment with both lithium and actinomycin D, also the latter may have an after-effect by its reaction with DNA. The transfer of information will at least be delayed, which tends also to hold back the regulation. The discrepancy between the above results and those of Lallier (1963) may not be regarded as removed by these suggestions. These writers have, however, considered that their experimental device would be most suitable to resolve the problem raised in the introduction. Furthermore detailed studies on the morphogenesis of the treated embryos, as well as measurements of incorporation of RNA-precursors, will be necessary in order to get more insight into the state of the embryos subjected to prolonged exposures.

The experimental part of this work was carried out at the Stazione Zoologica, Naples, during the spring of 1965. It is a pleasure to express our gratitude to the Director, Dr. Peter Dohrn, and to Dr. Luisa Tosi for their help and support. We acknowledge gratefully the financial help received from the Swedish Natural Sciences Research Council and the Swedish Cancer Society.

Summary

1. Embryos of *Paracentrotus lividus* were raised in sea water (C_0); in sea water with 12 μg./ml. actinomycin D (C_{act}); in sea water with 0.033 *M* lithium (Li_0) and in sea water with both 0.033 *M* lithium and 12 μg./ml. actinomycin D (Li_{act}). The embryos were transferred into the media at the 8-cell stage and exposed to these for 5.5 hours; thereafter they were returned to normal sea water. The embryos or larvae were fixed after different intervals of time as detailed in Table I. Living specimens were also observed. The lithium concentration and time of exposure used gave a weak but definite vegetalization in a high percentage of larvae (Table I, D, Li_0). The effect of lithium either involved merely a reduction of the animal-oral region (oral lobe) or was manifested also as an enlargement of the archenteron, in particular of its more animal (posterior) region. Accessory archentera or ectodermic bulges were often found in the border region between ecto- and entoderm. The differentiation seems here to be in a "fluctuating" state.

2. Previous results of Runnström, Ranzi and Lallier indicate that the primary effect of lithium increases inter- and intramolecular bondings in certain cytoplasmic components. The animalizing agents are assumed to be particularly sensitive to these changes which may block their diffusion. In this way, the normal animal-vegetal balance is disturbed in favor of the vegetalizing agents which are less affected by the structural changes.

3. The vegetalizing agents may directly or indirectly induce the activation of genes concerned with the vegetal anabolic pathways. When the action of these agents is enhanced in the state of unbalance caused by lithium, the predominance

of the vegetal anabolic pathways extends in animal direction. If, however, the transcriptions of genes are blocked by actinomycin D this extension of the region of vegetal pathways is inhibited (for a diagrammatic representation of lithium action on one side and the combined action of lithium and actinomycin D on the other, see Figure 13, Li_0 and Li_{act}). The blocking effect of actinomycin D does not depend on a direct reaction with the vegetalizing agents, as follows from data presented by Giudice and Hörstadius (1965). The inference is that gene activation is a link in the vegetalization following exposure to lithium.

4. Exposure to actinomycin D alone (Fig. 13, C_{act}) causes a tendency to animalization which is a secondary effect of retarded gastrulation. The development of the embryos becomes more balanced after exposure to the combined action than after that to actinomycin alone. A weak lithium effect balances the slight tendency to animalization which follows upon treatment with actinomycin D. Even after combined treatment, however, a certain inhibition prevails, manifested particularly in subnormal outgrowth of skeleton rods and arms.

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