Lithium as a Tool in the Analysis of Morphogenesis in *Limnaea stagnalis*

By CHR. P. RAVEN[1], Utrecht

Ten years ago it was discovered that a treatment with lithium chloride, which had proved to be so valuable in the analysis of development of sea urchins and amphibia[2], likewise gave rise to very interesting abnormalities of development in *Limnaea*[3]. A great number of experiments have since been made in our laboratory aiming at a further elucidation of this lithium action. It seems worth while to give a brief survey of these investigations, with the purpose clearly to define the present state of the problem.

The developmental abnormalities brought about by lithium in *Limnaea* are of two classes:

(1) exogastrulae, being spherical or dumb-bell shaped vesicles with a wall, partly consisting of ectoderm, partly of entoderm; and

(2) head malformations, mostly belonging to the cyclocephalic series: synophthalmia, cyclopia, anophthalmia, but including secondary deviations such as microphthalmia, monophthalmia, triophthalmia, asymmetries of the head and evaginations of the pharynx.

A study of 42 of these embryos with cyclocephalic head malformations[4] showed these malformations to be due to a suppression of the differentiation of mediodorsal head structures, leading to the fusion of the bilaterally situated "cerebral plates", which give rise to the cerebral ganglia, eyes and tentacles in normal development. From the fact that these mediodorsal head structures originate in normal development from the cells surrounding the animal pole of the egg, and that the most animal structures were most regularly suppressed, it was concluded that lithium in *Limnaea*, as in sea urchins[5], has a depressing action on a gradient-field with high point at the animal pole.

The study of exogastrulae produced by lithium treatment revealed that various kinds of cells in their walls may attain a certain degree of differentiation. Gut epithelium, entodermal albumen cells, stomodaeum and oesophagus cells, mesenchyme, larval kidney, ectodermal epithelium and big ectodermal ciliary cells may be distinguished. In general, the topographical relationships of these tissues correspond to the prospective significance of the cells in normal development. The entodermal tissues occupy the vegetative part of the exogastrulae, the ectoderm the animal part; the stomodaeum "anlage" and mesoderm form an equatorial girdle, which may be called the "marginal zone". However, in the ectodermal field a diversity exists in the topographical relationships of big- and small-celled ectoderm. Three types may be distinguished (Fig. 1). In type I, the small-celled ectoderm is present in the form of two areas ("cerebral plates"), surrounded on all sides by the big-celled ectoderm. This arrangement corresponds to that found in normal embryos. In type II, there is only one area of small-celled ectoderm, lying near the animal pole, and surrounded by a supra-equatorial girdle of big ectoderm cells. Finally, in type III no small-celled ectoderm at all can be found in the animal hemisphere, which is entirely composed of big ectoderm cells. Hence, these types represent a series of progressively increasing suppression of animal structures; they strongly support the hypothesis that one of the effects of lithium in *Limnaea* consists in the weakening of a gradient-field with high point at the animal pole.

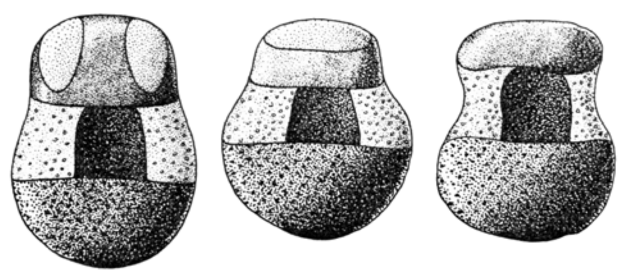

Fig. 1. – Three types of exogastrulae in *Limnaea*. From left to right: type I, II, III.

It must be emphasized that these changes in the pattern of determination brought about by lithium are restricted to the ectodermal hemisphere. No "entodermization" of the *Limnaea* exogastrula occurs; at most, some extension of the marginal zone towards the animal pole may take place. It must be assumed, therefore, that the animal gradient-field extends through the ectodermal region only or, at any rate, that the segregation only within this region takes place under the influence of the gradient-field.

[1] Zoological Laboratory, University of Utrecht.

[2] F. E. LEHMANN, *Einführung in die physiologische Embryologie* (Birkhäuser, Basel, 1945). – T. GUSTAFSON, Rev. suisse Zool. *57*, Suppl., 77 (1950).

[3] CHR. P. RAVEN, Proc. Kon. Ned. Akad. Wetensch., Amsterdam *45*, 856 (1942). (For the sake of brevity, these Proceedings will further on be quoted as "Proc.").

[4] CHR. P. RAVEN, Arch. néerl. Zool. *8*, 323 (1949).

[5] J. RUNNSTRÖM, Acta Zool. (Stockh.) *9*, 365 (1928).

In addition to this action on the animal gradient-field, lithium has an injurious action on the material of the vegetative hemisphere, which leads to exogastrulation or, with lesser injury to the cells, to disturbances in the invagination and the differentiation of the archenteron. Apparently, this effect is less specific than the suppression of the animalgradient-field. While head malformations as produced by lithium have only very seldom been obtained with other treatments, exogastrulae may appear in considerable proportions after centrifuging the eggs[1], under anaerobic conditions[2], and after treatment with KCl[3].

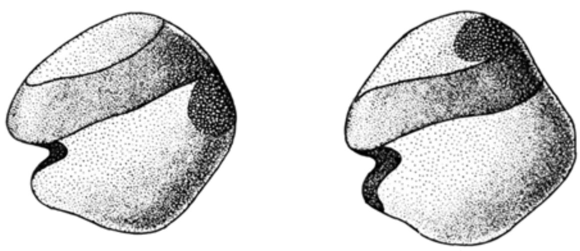

Fig. 2. – Induction of shell gland anlage (dark with white dots) in posttrochal ectoderm (left) and praetrochal ectoderm (right) of abnormal (type II) *Limnaea* embryos.

The inhibiting action of lithium on the vegetative material and its depressing influence on the animal gradient-field show no strict correspondence, since both exogastrulae without any appreciable reduction of animal differentiations (type I), and cyclopic or anophthalmic embryos with quite normal development of the intestinal tract, may occur. In general, however, both effects show a more or less parallel variation. This explains why cyclocephalic malformations are always found in a relatively low percentage only: most of the embryos, in which the depressing action on the animal gradient-field has been strongly pronounced, show gastrulation disturbances also and die at an early stage.

The study of totally and partially exogastrulated embryos revealed another important fact. In normal development, the shell gland forms as a thickening of the posttrochal ectoderm at the spot where the tip of the archenteron reaches the inner side of the ectoderm. For some days a very intimate contact between the shell gland "anlage" and the small-celled entodermal epithelium exists. In total exogastrulae no shell gland develops, but it may appear as soon as part of the entoderm has been invaginated and reaches the inner side of the ectoderm. The presumption that a contact induction plays a part was confirmed by the observation that in cases of abnormal gastrulation a shell gland may be induced in the praetrochal part of the ectoderm, if the tip of the archenteron comes to lie against this part (Fig. 2).

The Li-effects on the egg of *Limnaea* show a distinct phase-specificity. When the eggs are treated with 0·01% LiCl for one hour at various stages, there appears to be a distinct maximum of sensitivity for the production of exogastrulae at the 2- to 4-cell stage. On the other hand, the greatest number of head malformations developed when the eggs were treated either immediately after laying or at late cleavage stages[1]. Between these maxima there is a period, during which hardly any head malformations are produced with this treatment. With other combinations of LiCl concentration and exposure time, however, the maximum for the production of head malformations coincided with that for exogastrulae[2]. Moreover, our recent results, while showing that nearly 90% of the exogastrulae may exhibit a suppression of animal differentiations, have thrown another light on this phase-specificity. Apparently, both the maximum of sensitivity for exogastrulation and for the suppression of the animal gradient-field are to be placed at the 4-cell stage; most of the embryos treated at this stage die, however, before any head malformations become visible. Moreover, it seems that the lithium sensitivity of the animal gradient-field begins somewhat earlier and ends later than that of the vegetative material; this explains why hardly any exogastrulae, but only head malformations are produced by a treatment immediately after laying, while a treatment at advanced cleavage stages (12–32 cell stages) yielded only head malformations, but no exogastrulae at all[3]. This different course of the sensitivity curves forms another argument in favour of the essential difference between the lithium effects on the vegetative material and on the animal gradientfield.

Recent experiments indicate that the period of maximum sensitivity to lithium is but of very restricted duration. Theoretical considerations have led to the conclusion that the increase in concentration of lithium ions within the egg during treatment with these weak LiCl solutions must be rather slow, on account of the presence of the egg envelopes surrounding the egg; therefore, with short exposures, the maximum lithium concentration in the eggs will be reached only towards the end of treatment or even somewhat later. If this be so, then the sensitivity of the eggs at this moment must be of particular importance. As a matter of fact, it appeared that, especially with suboptimal combinations of concentration and exposure time, the numbers of exogastrulae and head malformations produced are dependent on the stage at which the treatment is ended and the eggs are returned to tap water[1].

[1] CHR. P. RAVEN and M. TH. C. VAN EGMOND, Proc. [C] *54*, 325 (1951).
[2] W. L. M. GEILENKIRCHEN, Proc. [C] *55* (in the press).
[3] P. F. ELBERS, Proc. [C] *55*, 74 (1952).

[1] CHR. P. RAVEN, J. C. KLOEK, E. J. KUIPER, and D. J. DE JONG, Proc. *50*, 584 (1947).
[2] CHR. P. RAVEN and M. A. SIMONS, Proc. *51*, 1232 (1948).
[3] CHR. P. RAVEN and A. H. G. C. RIJVEN, Proc. *51*, 437 (1948).

This may also explain the results of recent experiments, made with the purpose of studying the influence of temperature on the Li-effects[1]. The eggs were treated with 0·01% LiCl for one hour at 15°, 20°, 25° and 30°C, respectively. Curiously enough, no unequivocal influence of temperature could be established, because the two series of experiments yielded quite opposite results. In a first series, treatment was started at a middle 2-cell stage (stage 6–7)[2]. With increasing temperatures, the numbers of exogastrulae and head malformations and the mortality increased. On the contrary, in the second series, in which treatment was begun at a late 2-cell stage (stages 8–10), the numbers of exogastrulae, head malformations and dead embryos decreased with increasing temperatures. This discrepancy between both series could be explained, when allowance was made for the fact that the rate of development at various temperatures was quite different, so that the stage of development reached at the end of treatment differed among the treatment groups. In this way, it could be proved that the effects of the treatment were greatest when treatment ended at stage 11 (early 4-cell stage), and showed a regular decrease both before and after this stage. It is likely, therefore, that the sensitivity of the eggs reaches a maximum at stage 11 or (taking into account that it will take some time before the Li-contents of the eggs begin to decrease during rinsing after treatment) slightly later.

This conclusion is corroborated by other experiments, in which the influence of the egg-mass jelly on the Li-effects was studied[3]. Egg capsules were treated for 1 hour, from stage 6–7 to 10–11, with 0·01% LiCl, either enclosed in the jelly or after removal of the latter. After treatment they were rinsed in tapwater, again either with or without jelly. It appeared that the presence of the jelly during treatment had no influence on the Li-effects, but during rinsing it had: the eggs rinsed with the jelly gave a much greater percentage of exogastrulae and dead embryos than those rinsed without jelly. As the presence of the jelly will probably delay the washing-out of the lithium ions from the egg, but leading to a substantial difference between both groups only some time after the beginning of rinsing, again the conclusion seems justified that the lithium ion concentration in the eggs during a short period some time after stage 11 is most essential.

Finally, it may be mentioned that the shape of the sensitivity curve seems not to be identical for different concentrations of LiCl[4]. However, these relationships need further study; perhaps their analysis will throw more light on the whole problem of phase-specific lithium sensitivity.

1 A. C. J. BURGERS (in preparation).

2 Stages according to CHR. P. RAVEN, Arch. néerl. Zool. *7*, 353 (1946).

3 W. L. M. GEILENKIRCHEN, Proc. [C] *55*, 192 (1952).

4 E. D. NIJENHUIS, Proc. [C] *54*, 537 (1951).

For our understanding of the manner in which the morphogenetic disturbances are brought about by lithium treatment, it is essential to know the direct effects of lithium on the structure of the eggs. Therefore, in a number of investigations the cytology of lithium-treated eggs has been studied. It appeared that lithium affects the various constituents of the eggs differently.

In the first place, it seems to promote the swelling of the nuclei. In the eggs treated immediately after laying, this leads to a precocious and reversible swelling of the egg chromosomes into karyomeres immediately after the extrusion of the first polar body[1]. It is remarkable that in these cases the sperm nucleus, which in normal development remains compact and inactive till after the completion of the second maturation division, likewise exhibits a precocious swelling, and migrates towards the egg karyomeres situated near the animal pole. Evidently a causal relationship exists between the swelling of the pronuclei and their mutual attraction. Furthermore, lithium may provoke a swelling of the pronuclei during the last part of the uncleaved stage[2], of the cleavage nuclei during the early phases of cleavage[3] and at the 24-cell stage[4].

Secondly, lithium acts on the cytoplasm by increasing its density and stainability in the sections, and reducing the size of the cytoplasmic vacuoles[5]. At first, this increase in density of the cytoplasm was interpreted as a dehydratation phenomenon, but more recent experiments have shown that the water content of the egg as a whole is not specifically reduced by lithium chloride solutions, apart from their osmotic effects[6]. As the increased swelling of the nuclei is, presumably, fully counterbalanced by the reduction in size of the vacuoles, the increased density of the cytoplasm must be due to a change in its submicroscopic structure, e. g. by an increased polymerization of fibrillar macromolecules or the formation of cross-connections between protoplasmic fibrils. This is in agreement with the classical observations of RUNNSTRÖM in sea urchins[7] and the more recent investigations by RANZI and co-workers[8]. However, while the Li-effect in sea urchins is described by RUNNSTRÖM

1 A. P. DE GROOT, Proc. *51*, 588, 752 (1948). – CHR. P. RAVEN and J. R. ROBORGH, Proc. *52*, 614 (1949). – E. VAN DEN BROEK and CHR. P. RAVEN, Proc. [C] *54*, 226 (1951).

2 CHR. P. RAVEN and J. R. ROBORGH, l. c. – E. VAN DEN BROEK and CHR. P. RAVEN, l. c.

3 CHR. P. RAVEN and W. VAN ZEIST, Proc. *53*, 601 (1950). – E. VAN DEN BROEK and CHR. P. RAVEN, l. c. – E. D. NIJENHUIS, l. c.

4 CHR. P. RAVEN and S. DUDOK DE WIT, Proc. *52*, 28 (1949).

5 CHR. P. RAVEN and W. VAN ZEIST, l. c. – E. VAN DEN BROEK and CHR. P. RAVEN, l. c. – E. D. NIJENHUIS, l. c.

6 CHR. P. RAVEN, J. J. BEZEM, and R. P. VAN LOO, Proc. [C[*55*, 7 (1952).

7 J. RUNNSTRÖM, Acta Zool. (Stockh.) *9*, 365 (1928).

8 S. RANZI, R. AROSIO, P. CITTERIO, P. MENOTTI, and F. SEMENZA, Exper. *2*, (1946). – P. CITTERIO and S. RANZI, Rend. Accad. naz. Lincei, Cl. Sci. (8) *3*, 150 (1947). – S. RANZI, P. CITTERIO and M. GIUDICI, Exper. *4*, 112 (1948).

as a coarsening of cytoplasmic structure, microincineration studies in *Limnaea* rather point to an increased dispersion at least of the inorganic constituents of the cytoplasm[1]. It is hoped that this point may be further elucidated by electron-microscopical investigations, which have been undertaken this year.

The general increase in density of the cytoplasm by lithium may account for some further Li-effects: a greater distinctness of astral radiations of the spermaster after the second maturation division, and occasional rotations of the first cleavage spindle in Li-treated eggs[2].

Other observations point, however, to a particular densifying action of lithium on the cortical cytoplasm. The displacement of the nuclei towards the surface at the 24-cell stage[3] may be due to this effect. Similarly, lithium brings about a displacement of the spindles of third cleavage towards the animal side, which leads to a reduction in size of the micromeres[4]. This effect becomes more pronounced with increasing concentrations of LiCl; with 0·1% LiCl, the relative sizes of the micromeres have diminished by no less than ⅓ of the normal volume.

A further group of deviations in egg structure after treatment with lithium belongs to the class of "depolarization phenomena" according to DALCQ[5]: formation of giant polar bodies, rotation of the second maturation spindle, displacement of egg karyomeres from the animal pole towards the centre of the egg, and delayed migration of the sperm nucleus[6]. As all these phenomena occurred only after treatment with isotonic or slightly hypertonic LiCl solutions and, moreover, similar deviations have been obtained with $CaCl_2$ solutions of the same osmotic pressures[7], one might think that the osmotic pressure of the solutions as such is responsible for the effects. However, with isotonic and hypertonic non-electrolyte solutions hardly any depolarization phenomena have been observed[8].

Finally, lithium influences the processes of ooplasmic segregation going on in the egg[9]. The distribution of the subcortical protoplasm is highly abnormal in an isotonic LiCl solution. The animal pole plasm does not form at all in LiCl solutions. When afterwards the eggs are transferred to tapwater and recovery takes place, the animal pole plasm may be formed with some delay[10]. When the eggs are treated at the 2-cell stage, the animal

[1] H. J. VAN DER LEE, unpublished observations.
[2] CHR. P. RAVEN and J. R. ROBORGH, l. c. – E. VAN DEN BROEK and CHR. P. RAVEN, l. c.– Cf. CHR. P. RAVEN, J. J. BEZEM and J. ISINGS, Proc. [C] *55* (in the press).
[3] CHR. P. RAVEN and S. DUDOK DE WIT, l. c.
[4] CHR. P. RAVEN, J. J. BEZEM, and J. ISINGS, l. c.
[5] A. DALCQ, Arch. Biol. *34*, 507 (1925).
[6] A. P. DE GROOT, l. c.
[7] CHR. P. RAVEN and J. C. A. MIGHORST, Proc. *49*, 1003 (1946).
[8] CHR. P. RAVEN and W. HUPKENS VAN DER ELST, Proc. *53*, 1005 (1950).
[9] A. P. DE GROOT, l. c.
[10] E. VAN DEN BROEK and CHR. P. RAVEN, l. c.

pole plasm, which has been formed at this time, does not disappear[1]. At later cleavage stages, irregularities in the distribution of plasm substances among the cells have often been observed.

Many experiments have been made in order to study the physiology of Li-action in *Limnaea*. A comparison with the influence of NaCl, KCl, $MgCl_2$ and $CaCl_2$ solutions showed that both exogastrulae, and head malformations may be produced in a few cases by the other salts as well, but their number is much greater with LiCl. These malformations are, therefore, relatively specific Li-effects[2]. As mentioned above, this holds especially for the head malformations, whereas exogastrulae may be produced in various ways. The number and nature of malformations induced by LiCl is not very dependent on concentration and exposure time.

When eggs are treated with hypertonic LiCl solutions, development stops the earlier, the more the solution is concentrated[3]. A degeneration of maturation spindles occurs, or, in weaker solutions, a more or less abortive cleavage may take place. These effects are all due to hypertonicity as such as they also occur in solutions of non-electrolytes of similar osmotic pressures[4].

More interesting is the fact that decapsulated eggs in hypotonic LiCl solutions do not develop beyond the prophase of 3rd cleavage[5]. The same holds for eggs in distilled water; but, while the block of development at this stage may be broken by the addition of NaCl, KCl, $MgCl_2$ or $CaCl_2$ at certain concentrations, LiCl in none of the concentrations used with certainty gives this effect. It is interesting to note that this stage, at which development comes to a standstill in distilled water and lithium chloride solutions, about coincides with the stage of maximal sensitivity to lithium. Moreover, at this stage the eggs are also highly susceptible to the action of urea[6], thiourea[7] and centrifugal force[8].

The changes in viscosity and tension at the surface, brought about by lithium chloride, have been studied by means of the centrifugation method[9]. A decrease in viscosity seemed to take place in stronger LiCl solutions (0·1% and higher). However, in this investigation the evaluations of viscosity were especially based on the occurrence of vacuoles and granules in the hyaloplasm zone. As we now know, the swelling of the γ-granules into vacuoles[10] and, by this,

[1] CHR. P. RAVEN and W. VAN ZEIST, l. c.
[2] CHR. P. RAVEN and M. A. SIMONS, l. c.
[3] A. P. DE GROOT, l. c.
[4] CHR. P. RAVEN and W. HUPKENS VAN DER ELST, l. c.
[5] M. S. GRASVELD, Proc. *52*, 284 (1949). – CHR. P. RAVEN and W. VAN ZEIST, l. c.
[6] CHR. P. RAVEN and H. KLOMP, Proc. *49*, 101 (1946).
[7] F. H. SOBELS, Proc. *51*, 900 (1948).
[8] CHR. P. RAVEN and M. TH. C. VAN EGMOND, l. c.
[9] G. A. DE VRIES, Proc. *50*, 1335 (1947).
[10] CHR. P. RAVEN and L. H. BRETSCHNEIDER, Arch. néerl. Zool. *6*, 255 (1942). – CHR. P. RAVEN, Arch. néerl. Zool. *7*, 91 (1945).

their accumulation in the centripetal half of the egg at centrifuging, is inhibited by lithium. Therefore, it is probable that the decrease in viscosity at higher LiCl concentrations described in this paper is only apparent. This does not hold for the slight, but apparently significant increase in overall-viscosity observed with weaker (0·006–0·01%) LiCl solutions. The tension at the surface was increased by hypertonic LiCl solutions, somewhat decreased by moderately hypotonic (0·1%) solutions.

The latter solutions also cause an increase in water permeability of decapsulated *Limnaea* eggs[1].

Finally, an increase in amoeboid motility of the eggs has been observed especially in hypertonic[2], but also in isotonic and moderately hypotonic LiCl solutions[3].

The physical properties of the vitelline membrane and egg cortex in *Limnaea* are greatly dependent on the ionic composition of the surrounding medium. In distilled water and $Ca^{\cdot\cdot}$-free solutions the vitelline membrane is thrown into folds; the first polar body, which is tightly surrounded by the membrane in normal eggs, under these circumstances comes to lie in the space beneath the vitelline membrane. During cleavage, the membrane bridges the cleavage furrows and soon loses contact with the surface of the cells. The blastomeres do not flatten themselves against each other and no cleavage cavity is formed[4]. It was concluded from these observations that the consistency and adhesiveness of the vitelline membrane and the physical properties of the egg cortex are changed in the absence of $Ca^{\cdot\cdot}$-ions. However, as regards the egg cortex, the part of calcium may to some extent be taken over by lithium: in moderately hypotonic LiCl solutions cleavage, as far as it takes place, proceeds rather normally, with flattening of the blastomeres and formation of a cleavage cavity[5]. In NaCl cleavage type is less normal, in KCl it is very abnormal.

Ionic equilibria also seem to play an important part in the causation of the morphogenetic Li-effects. Li-solutions in distilled water have a much stronger effect than solutions in tapwater. The former yielded the largest number of head malformations at a concentration of $2{\cdot}16 \times 10^{-5}$ *M* with a treatment of 24 hours; for exogastrulae, the optimum concentration was $4{\cdot}8 \times 10^{-5}$ *M*[6]. Stronger solutions in distilled water soon give 100% mortality, whereas the same concentrations in tapwater are much less harmful.

Contrary to what has been found in sea urchins[1], however, potassium has no antagonistic action with respect to lithium, but even intensifies the morphogenetic effects of LiCl, as it does in Amphibia[2].

There are other indications that physiology of lithium action in *Limnaea* differs from that in sea urchins. A diminution in the partial oxygen pressure does not increase the lithium effects, as in sea urchins[3]; under anaerobic conditions the characteristic head malformations even do not appear at all[4]. Evidently, the suppression of the animal gradient-field is in some way or other dependent on the presence of oxygen. Likewise, lactic acid does not reinforce the morphogenetic action of lithium[5], as it does in sea urchins[6].

Although a large number of data on lithium action in *Limnaea* have been obtained in these investigations, it is not yet possible to piece them together into a coherent picture of the causation of morphogenetic disturbances by lithium action. Especially, no direct connection could be established between the direct, cytological effects of lithium treatment and its indirect, morphogenetic effects, though some investigations have been made in which both groups of phenomena have been studied side by side in the same material[7].

Up to the present, the most obvious hypothesis seems to be that lithium interferes in some way or other with the displacements of ooplasmic substances which lead in normal development to their arrangement along a system of gradients. This might account both for the gastrulation disturbances and for the suppression of animal differentiations. As a matter of fact, an abnormal distribution of the subcortical plasm and a suppression of the formation of the animal pole plasm by lithium has been observed, as recorded above. It has been shown that the accumulation of the animal pole plasm in normal development is due to attractive actions exerted locally by the egg cortex[8]. Lithium, which obviously influences the properties of the cortex (cf. above), may inhibit these cortical attractions, and lead, in such a way, to an abnormal distribution of egg substances. In view of the great importance which has lately been attached to the ribonucleic acids in vertebrate development[9], the distribution of these substances in uncleaved lithium-treated *Limnaea* eggs has been investigated, but no abnormalities have been

1 CHR. P. RAVEN, J. J. BEZEM, and R. P. VAN LOO, l. c.

2 A. P. DE GROOT, l. c. – M. S. GRASVELD, l. c. – G. BLAAUW-JANSEN, Proc. *53*, 910 (1950).

3 CHR. P. RAVEN and J. R. ROBORGH, l. c.

4 CHR. P. RAVEN and H. KLOMP, l. c. – O. HUDIG, Proc. *49*, 554 (1946). – M. S. GRASVELD, l. c.– TH. G. J. STALFOORT, Proc. [C] *55*, 184 (1952).

5 A. P. DE GROOT, l. c. – M. S. GRASVELD, l. c. – CHR. P. RAVEN and W. VAN ZEIST, l. c.

6 P. F. ELBERS, l. c.

1 J. RUNNSTRÖM, Acta Zool. (Stockh.) *9*, 365 (1928). – P. E. LINDAHL, Acta Zool. (Stockh.) *17*, 179 (1936.

2 F. E. LEHMANN, Naturwissenschaften *25*, 124 (1937).

3 P. E. LINDAHL, Roux'Arch. *140*, 168 (1940).

4 W. L. M. GEILENKIRCHEN, l. c.

5 G. SCHOLTEN, Proc. [C] *55*, 203 (1952).

6 P. E. LINDAHL (1940), l. c.

7 E. VAN DEN BROEK and CHR. P. RAVEN, l. c. – E. D. NIJENHUIS, l. c.

8 CHR. P. RAVEN, Arch. néerl. Zool. *7*, 91 (1945). – CHR. P. RAVEN and F. BRUNNEKREEFT, Proc. [C] *54*, 440 (1951).

9 Cf. J. BRACHET, *Le rôle des acides nucléiques dans la vie de la cellule et de l'embryon* (Actualités biochimiques, Liège/Paris, 1952).

observed[1]. Curiously enough, in one group of experiments it appeared that the number of lithium malformations in different batches varied inversely with the degree of abnormality in the distribution of the animal pole plasm[2].

The above-mentioned hypothesis finds support in the fact that the period of maximum sensitivity to lithium at the 4-cell stage coincides with a phase, in which important processes of ooplasmic segregation take place, leading to a condensation of dense cytoplasm at the animal pole prior to 3rd cleavage[3].

At this moment, the eggs show also a maximum sensitivity to the effects of centrifuging[4]. The fact that, by centrifugation at this moment, abnormalities of development may be produced which show a certain resemblance to lithium malformations, is a further support for the view that both treatments have their point of attack in the same developmental processes. This is corroborated by the observation that after combined centrifugation and lithium treatment a mere addition of the effects of both single treatments results, without any indication of a more complex interaction[5]. Furthermore, the earliest visible signs of abnormality in eggs treated with weak LiCl solutions are just such changes in the arrangement and proportions of cytoplasmic substances as might be expected to be caused by their abnormal distribution at early cleavages.

Finally, an important phase for the determination of the egg may be the 24-cell stage. At this stage, cleavage divisions stop for 5–6 hours; all cells extend with clear prolongations towards the centre of the egg, where they meet. Apparently, a transfer of substances (glycogen, ribonucleic acid) between the animal and vegetative cells takes place[6]. At the same time, this stage is the last one at which changes in the pattern of head organs may be brought about by lithium treatment[1]; the determination of this pattern evidently takes place at this moment or shortly afterwards. This has led MINGANTI to assume that a contact induction between the vegetative and the animal cells takes place at this stage, which is attended with a transfer of ribonucleic acid from the former to the latter. As a matter of fact, centrifuging the eggs for some hours at the 24-cell stage is much more harmful than at the 16-cell stage[2]; this might be due to an interference with the transfer of substances from cell to cell. Again it might be supposed that lithium acts in the same way, by interfering with the displacement of substances (ribonucleic acid bound to microsomes?) from the vegetative towards the animal cells. However, a study of lithium-treated eggs at the 24-cell stage with BRACHET's cytochemical technique for ribonucleic acid did not reveal any abnormality in the distribution of this substance[3].

We may conclude that the manner in which lithium exerts its influence on the development of *Limnaea* is clearly indicated, but further research will be needed before a comprehensive picture of the concatenation of causes and effects can be drawn.

Zusammenfassung

Die Analyse der von Lithiumchlorid im *Limnaea*-Keim erzeugten Missbildungen hat gezeigt, dass in der Entwicklung dieser Art ein animales Gradientfeld mitspielt, das die Abgrenzung der Anlagen im ektodermalen Gebiet beherrscht. Auf späterem Stadium wird die Schalendrüse im Ektoderm durch eine Kontaktinduktion von seiten des Urdarms induziert. Die Lithiumwirkung ist phasenspezifisch; wahrscheinlich gibt es eine sehr kurze Periode maximaler Empfindlichkeit gegen Ende des vierzelligen Stadiums. Lithium verursacht eine Schwellung der Kerne und eine Verdichtung des Zytoplasmas, besonders des kortikalen Plasmas; die ooplasmatische Segregation ist gestört. Weiterhin beeinflusst es die physikalischen Eigenschaften des Plasmas. In physiologischer Hinsicht weicht die Lithiumwirkung bei *Limnaea* in einigen Punkten von der bei Seeigeln ab.

[1] Miss J. E. H. SMIT, unpublished observations.
[2] E. VAN DEN BROEK and CHR. P. RAVEN, l. c.
[3] CHR. P. RAVEN, Arch. néerl. Zool. 7, 353 (1946).
[4] CHR. P. RAVEN and M. TH. C. VAN EGMOND, l. c.
[5] Miss TH. C. M. KOEVOETS, unpublished observations.
[6] CHR. P. RAVEN, Arch. néerl. Zool. 7, 353 (1946). – A. MINGANTI, Riv. Biol. 42, 295 (1950).

[1] CHR. P. RAVEN and A. H. G. RIJVEN, l. c.
[2] F. J. VERHEIJEN, unpublished observations.
[3] G. A. VAN ARKEL, unpublished observations.