Zoology. — *The influence of higher concentrations of lithium chloride on maturation and first cleavages of the egg of Limnaea stagnalis.* II.
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(Communicated by Prof. CHR. P. RAVEN.)

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3. *Influence on cleavage.*

RAVEN and KLOMP (1946) showed that the lack of Ca^{++}-ions in the medium prevents a normal flattening of the blastomeres after the first cleavage, the result of which is a loosening of the vitelline membrane from the egg cortex. A normal cleavage was obtained by adding a small quantity of $CaCl_2$ to the medium.

As mentioned above, in certain concentrations of LiCl not only a first cleavage occurs, but even the blastomeres may flatten in a normal way, leading to the formation of a cleavage cavity in 0.1 and 0.05 LiCl. The presence of small quantities of $CaCl_2$ cannot be the cause of this. 1) The adhering capsule fluid was carefully removed from the egg surface by repeated washings. Eggs transferred to distilled water, after washing less intensively, developed abnormally. 2) If broken eggs were removed from the solution, normal cleavage still occurred. 3) The presence of some Ca as contamination in the LiCl employed cannot be excluded with certainty, but surely not in such a quantity as would be necessary to account for the effects. According to RAVEN and KLOMP (1946) an appreciable effect of $CaCl_2$ on cleavage can only be obtained with solutions of 0.005 % or higher. So, if Ca should be the cause of the normal flattening of the blastomeres in the LiCl solutions, a contamination of 10 % Ca would be necessary. This possibility may be left out of account. From the above considerations it may be concluded that besides $CaCl_2$ also LiCl is able to cause a flattening of the blastomeres after the first cleavage.

O. HUDIG (1946) found a normal cleavage of the *Limnaea* egg in KCl and in Na-citrate as well. In the former solution it was already observed by PASTEELS (1930) who treated the eggs of *Barnea candida*. PLOUGH (1927) observed a normal first cleavage of *Arbacia* eggs in seawater after precipitating the Ca with the aid of citrate. Thus in $CaCl_2$, LiCl, KCl and Na-citrate a normal first cleavage may occur.

RAVEN and MIGHORST (1946) found that a morula stage may be reached in distilled water after a temporary treatment of *Limnaea* eggs in $CaCl_2$. In the present investigation several egg-masses were treated in the same way with LiCl. It was observed that after a temporary treatment and subsequent transfer to distilled water, a cleavage might occur, whereas it was suppressed if the eggs remained in the employed concentration of LiCl;

after that development stopped, however. Probably, this discrepancy between the Li- and Ca-effect is due to a particular function of the Ca^{++}-ions in development. From the experiments of RAVEN and KLOMP (1946) it was concluded that Ca^{++}-ions affect the properties of the vitelline membrane. The investigation of O. HUDIG (1946) led to the conclusion that the absence of Ca^{++}-ions alters the egg cortex as well. In the present investigation it was noted that after transfer to LiCl the eggs stuck to the bottom of the containers. This occurred especially after removing the adhering capsule fluid by washing in distilled water. It is likely, therefore, that the outer layer of the egg, i.e. the vitelline membrane, is affected by LiCl.

As was already mentioned, in 0.05 % the first cleavage was completely normal. In higher concentrations, the cleavage is less normal or suppressed altogether, whereas in distilled water the blastomeres do never flatten. So there exists an optimal favourable concentration of LiCl with respect to segmentation.

4. *Inhibitory action.*

The observations described above show the possibility of stopping the processes of maturation and fertilization at various stages.

a. In 1.0 % LiCl, development may stop at the stage of the first maturation spindle even before the first maturation division. This was shown by the sectioned eggs. Since the *Limnaea* egg is deposited at a stage with the first maturation spindle in metaphase, the development must have been stopped nearly immediately after exposing the egg to this high concentration. The deviations of treated eggs, as compared with normal ones, are, apparently, of a degenerative nature only.

b. The inhibitory action of LiCl may bring the development to a standstill immediately after the first maturation division. This happened in several eggs in 0.5 and 0.4 %.

c. In 0.4 %, in some of the eggs a second maturation spindle was formed; however, a second polar body may not be extruded.

d. Other eggs in the same concentration were inhibited after the formation of the second polar body, showing karyomeres beneath the egg cortex or near the centre of the egg.

e. Finally, in 0.4 % a copulation of the pronuclei may occur. This was the most advanced stage, observed in this concentration. In spite of the large number of eggs, a first cleavage was never observed.

5. *Depolarization phenomena.*

A number of the above-mentioned abnormalities may certainly be considered as belonging to the group of phenomena called "depolarization" by DALCQ (1925).

a. The most fascinating manifestation of depolarization is certainly the displacement of the second maturation spindle from the animal pole, followed by a rotation of 180°, in such a way that the spindle places itself perpendicularly to the egg axis. RAVEN and MIGHORST (1946) observed this condition in a considerable number of eggs treated with 0.5 % $CaCl_2$. In the present investigation the same phenomenon occurred only in a few eggs. This may possibly be explained by the small percentage of eggs, which reached the stage of the second maturation spindle. Only in one out of three egg-masses, sectioned after treatment with 0.4 % LiCl, the rotation of the second naturation spindle was observed. This egg-mass had been transferred to the solution at a later stage than the other ones and showed the least disturbances. So a more frequent occurrence of a rotated maturation spindle may be expected in a somewhat lower concentration, or in the same solution if the treatment starts at a somewhat later stage of development.

b. If the position of the second maturation spindle is not affected and a second polar body is formed, a depolarization may become visible in an abnormal position of the karyomeres. Normally these are lying close beneath the egg cortex at the animal pole. In most of the eggs, sectioned after treatment with 0.4 % LiCl, the karyomeres were situated at a certain distance from the egg cortex, in some of them even in the centre. The attractive mechanisms, normally determining the position of the karyomères, seem to be weakened by the LiCl treatment.

c. According to DALCQ (1925) and PASTEELS (1930), the enlargement of polar bodies has to be considered as a depolarization phenomenon too. In the present investigation, polar bodies which easily could be recognized as giant polar bodies, occurred in concentrations of 0.2 up to 0.4 %, although in a very small number. The normal volume was surpassed several times by about 2.5 % of the observed polar bodies only.

d. Finally, the delayed migration of the sperm nucleus must be mentioned. Since the polarity of the egg must play a part in the normal displacement of the sperm nucleus to the animal pole, it is very likely that the inhibition of this phenomenon is caused by a disturbance of the attractive factors. Possibly, the accelerated migration observed in a number of eggs points in the same direction. We are inclined to classify both inhibition and acceleration as depolarization phenomena.

Possibly, more abnormalities are to be considered in the same way. As the polarity plays such an important part in maturation and segmentation, it is clear that damaging influences, although of a different nature, may cause deviations in all those processes which normally are determined by the polarity, i.e. depolarization. It is not likely that the rotation of the second maturation spindle has a particular place among these phenomena. The great variety of influences resulting in a rotation points in that direction. Not only $CaCl_2$ and LiCl, but also hypertonicity, hypotonicity, CO_2 and pure mechanical pressure (KING 1906) may cause this rotation. We are inclined to consider a depolarization phenomenon not as a special reaction to a special stimulus, but rather as an indication that a certain event is dependent on polar factors acting in a developing egg.

6. *Migration of the sperm nucleus.*

A number of eggs treated with 0.4 % LiCl showed a remarkable behaviour of the nuclear apparatus. About 30 min. before the second maturation division in the controls, the chromosomes had already developed into karyomeres, situated close to the egg cortex at the animal pole, in the remnants of the first maturation aster. At this moment the development of the sperm nucleus into a male pronucleus had already proceeded very far, and its migration to the animal pole had already started or was even completed. Normally the sperm nucleus retains its subcortical position and its original shape until about 20 min. after the completion of the second maturation division. Thus an acceleration, amounting to 1—1½ hours, had happened. Apparently, after the extrusion of the first polar body, a condition had been reached, which normally does not occur before the end of the second maturation division. The LiCl treatment seems to have activated at an early hour the directing factors acting upon the sperm nucleus. This effect of the LiCl treatment may be considered as belonging to a class of phenomena, called "mise à l'unisson" by BRACHET (1922): the sperm nucleus passes prematurely into a stage resembling that of the egg nucleus. An opposite effect was observed in other eggs, treated with the same solution. Here the moment of migration of the sperm nucleus was considerably delayed. In these cases, the eggs showed subsided egg-karyomeres. The factors responsible for the ascent of the sperm nucleus seem to be inhibited in this case. Possibly, this simultaneous effect on both egg karyomeres and sperm nucleus points to a relation between their positions. Moreover, it supports the view that the subsidence of egg kayomeres may be considered as a depolarization phenomenon.

7. *Cytoplasmic effects.*

The LiCl treatment does not only affect the nuclear processes, but also the cytoplasmic components. The structure of the cytoplasm is changed especially in higher concentrations, showing a more compact appearance. In concentrations more or less isotonic to the egg, this influence was not visible. Probably, it is the result of a withdrawal of water by hypertonicity of the medium.

The animal pole plasm, occurring normally one hour before the first cleavage, had been formed in none of the sectioned eggs. Only in a very few cases a doubtful indication of it was observed. Its formation is, apparently, suppressed by the LiCl treatment.

The subcortical plasm is influenced in a peculiar way; this effect is the more interesting owing to the concentration in which it is most pronounced. In a large number of sectioned eggs the subcortical plasm had not spread beneath the egg cortex in a normal way. Its distribution was most abnormal in eggs treated with 0.2 %. In a less degree, the abnormal situation occurred also in 0.4 and 0.5 % solutions, whereas in 1.0 % hardly any abnormality was visible. In the higher concentrations it had spread in a rather normal way, showing only unimportant accumulations at various places. In 0.2 %, however, a normal distribution was never observed. It is piled up at one or more places, even at the animal pole. With proceeding development of the egg the distribution becomes more regular and at the two-cell stage it is only slightly irregular.

Whereas nearly all the above-mentioned abnormalities can be ascribed to the hypertonicity of the solutions employed, it is not allowed to attribute the abnormalities of the subcortical and animal pole plasm to the same factor. The abnormal distribution of the subcortical plasm is most pronounced in an isotonic solution and, hence, cannot be due to hypertonicity. The suppression of the animal pole plasm happens in each concentration. So the specific properties of LiCl are to be adduced to explain these abnormalities.

In contrast with the abnormal behaviour of the animal pole plasm and subcortical plasm, the maturation divisions and the first cleavage did not show any irregularitie in about isotonic concentrations. From this we are forced to conclude that the nuclear cycle is disturbed especially by hypertonicity, whereas in more or less isotonic solutions of LiCl the cytoplasmic components of the egg are particularly affected. Further experiments will be needed to test this hypothesis.

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Summary.

1. Eggs of *Limnaea stagnalis* were treated shortly after oviposition with 12 different concentrations of LiCl, varying from 4.0 % to 0.05 % (osmotic pressure: 42.2—0.5 atm.).
2. The development does not proceed further than the second cleavage. It may be inhibited at various stages, dependent on concentration, stage of treatment, temperature and susceptibility of the eggs.
3. Above a certain concentration of the medium, all eggs orient with the animal pole downwards; probably, this is due to local differences in permeability of the egg cortex.
4. Abnormalities, considered as depolarization phenomena, were observed in concentrations between 0.5 and 0.2 %
5. In hypotonic solutions the first cleavage may be completely normal. From this it was concluded that LiCl may prevent the loosening of the vitelline membrane from the egg cortex.
6. The nuclear cycle of maturation and fertilization is disturbed in hypertonic solutions only, whereas the animal pole plasm and the

subcortical plasm show abnormalities in their distribution even in isotonic concentrations. Therefore, it is likely that LiCl exerts a specific influence on the cytoplasmic components of the *Limnaea* egg.

REFERENCES.

BRACHET, A., Arch. Biol. **32**, 205 (1922).

DALCQ, A., Arch. Biol. **33**, 79 (1923).

———, Arch. Biol. **34**, 507 (1925).

HERBST, C., Z. wiss. Zool. **55**, 446 (1893).

HUDIG, O., Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **49**, 554 (1946).

KING, H. D., Arch. f. Entw. Mech. **21**, 94 (1906).

LEHMANN, F. E., Roux' Arch. **136**, 112 (1937).

LEPLAT, G., Arch. Biol. **30**, 231 (1920).

PASTEELS, J., Arch. Biol. **40**, 247 (1930).

RAVEN, CHR. P., Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **45**, 856 (1942).

———, Arch. néerl. zool. **7**, 91 (1945).

RAVEN, CHR. P. and L. H. BRETSCHNEIDER, Arch. néerl. zool. **6**, 255 (1942).

RAVEN, CHR. P. and H. KLOMP, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **49**, 101 (1946).

RAVEN, CHR. P. and J. C. A. MIGHORST, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **49**, 1003 (1946).