

ZOOLOGY

INDUCED ROTATION OF CLEAVAGE SPINDLES IN LIMNAEA STAGNALIS L.

BY

J. FABER

(Zoological Laboratory, University of Utrecht)

(Communicated by Prof. CHR. P. RAVEN at the meeting of Sept. 30, 1950)

Introduction:

The present investigation was induced by the work of F. H. SOBELS (1948). During his investigations on the influence of thiourea on the development of *Limnaea stagnalis* he observed occasional rotations of cleavage spindles, especially when decapsulated eggs were treated with a 0.75 % thiourea solution.

Similar phenomena have been mentioned by HÖRSTADIUS (1928) in eggs of *Paracentrotus lividus*, after shaking or meridionally cutting the eggs and even after exposure to diluted seawater, and by CONKLIN (1938) in eggs of *Crepidula plana* after treatment with low temperatures. Both authors suggest the possibility that the factors governing the direction of cleavage spindles are relatively independent from the other developmental processes, and may cause the cleavage spindle to assume the correct position according to the age of the egg, even if one or more cell divisions or even nuclear divisions did not occur due to any form of retardation of development. This hypothesis explains e.g. the often observed so-called "premature micromere-formation", in which, in spiral cleavage, micromeres are formed, not from a 4-blastomere stage, but from a 2-blastomere or even an uncleaved stage. HÖRSTADIUS has been able to explain nearly all the abnormal cleavages experimentally obtained by him with this hypothesis, combined with the known facts about the micromere-forming potencies of the vegetative plasm of the Echinoderm egg. The hypothesis can also be applied to the greater part of CONKLIN's abnormal cleavages, but in a number of cases the abnormalities are so irregular that he is forced to assume a direct disorienting effect of the cold treatment on the cleavage pattern.

The same hypothesis has been applied by SOBELS (1948) to the spindle rotations observed in *Limnaea*. The purpose of the present investigation has been, therefore, to give an answer to the following questions:

1. Is spindle rotation always coupled with retardation of development?
2. Does spindle rotation occur more or less simultaneously with the normal changes in the direction of cleavage spindles in the control eggs?

Methods:

Egg-masses were obtained by stimulating the snails with *Hydrocharis*, as indicated by RAVEN and BRETSCHNEIDER (1942).

Only decapsulated eggs were used and thiourea was applied in a concentration of 0.75 %. As a solvent a solution of 0.04 % CaCl_2 was used, in order to provide the necessary Ca^{++} -ion concentration (HUDIG 1946).

The eggs were observed in the experimental solution till after the 3rd cleavage; the only variable factor has been the moment at which they were put into the solution. The control eggs were kept in a 0.04 % CaCl_2 solution. All eggs were kept at a constant temperature of 23° C.

In addition to the experiments with thiourea a number of eggs were treated with different concentrations of sucrose, dissolved in 0.04 % CaCl_2 , and some experiments concerning the influence of low temperatures were carried out.

Experimental results:

A. Thiourea

1. *Abnormalities and types of rotation*

a. In general we observe that the blastomeres are always more rounded than in normal development, which makes it impossible to establish exactly the stage of development according to RAVEN's normal table (1946).

b. *First cleavage.* An equatorial cleavage, often observed by CONKLIN in *Crepidula*, never occurred in these experiments. Rotation of the first cleavage spindle apparently occurs only in cases in which inhibition is so strong that the egg remains uncleaved¹⁾ (cf. SOBELS, p. 905).

c. *Second cleavage.* In most of the rotation-types described, rotation occurs in a plane parallel to the original plane of the first cleavage. It is impossible to tell the direction of the rotation, as in most cases it is unknown which pairs of blastomeres originate from the same $\frac{1}{2}$ -blastomere.

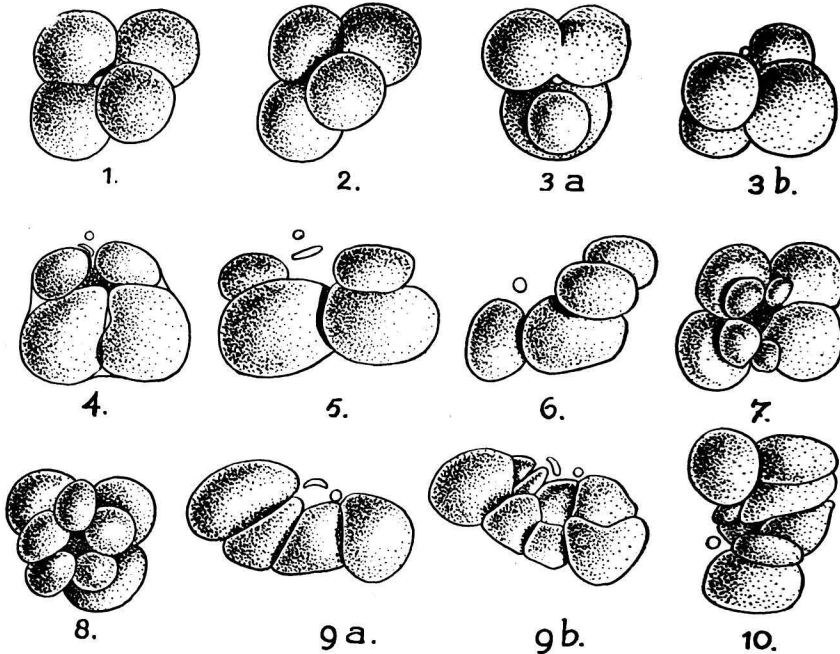
A series of separate types, established according to the rotation-angle, is illustrated by figs. 1—4. The type of fig. 1 is reversible; it consists, at most, of a slight exaggeration of the normal obliquity of the second cleavage spindles, and has, therefore, not been considered a rotation in the analysis of the experimental figures. The types of figure 2, 3 and 4 will henceforth be indicated as I, II and III. Type I (fig. 2) is formed by eggs, in which one of the blastomeres is distinctly lying above the plane of the 3 other ones. Type II is the rotation described by SOBELS, in which one of the blastomeres divides nearly meridionally and the other one in an equatorial plane²⁾. In type III both blastomeres apparently divided

¹⁾ In *Crepidula* the case is similar, as the equatorial two-cell stages seldom develop any further (cf. CONKLIN, p. 188—189).

²⁾ As to the origin of type II it is remarkable that I never, in about 20 observations of this type, observed it to originate from a 3-cell stage, in the way SOBELS described.

equatorially, as indicated by the position of the polar bodies. It is observed often, though not in all cases, that the animal blastomere of a rotated pair is smaller, the vegetative one bigger than the blastomeres of the non-rotated pair.

Type III has some variants, which prove that the planes of rotation are not always parallel to the first cleavage plane (fig. 5)³⁾, whereas the positions of the second cleavage planes may vary (cf. fig. 9a in which the



Limnaea stagnalis. Abnormal cleavage stages.

- Fig. 1. Exaggeration of obliquity of second cleavage spindles.
 Fig. 2. Rotation-type I.
 Fig. 3. Rotation-type II.
 Fig. 4—6. Rotation-type III.
 Fig. 7—8. Abnormal 8-cell stages derived from normal 4-cell stages.
 Fig. 9. Third cleavage of type-III egg.
 Fig. 10. Eight-cell stage derived from type-III egg.

animal blastomeres are bigger than the vegetative ones). The thoroughly disorienting effect of thiourea on the second cleavage is most evident in a type as shown in fig. 6, and in the T-shaped eggs, observed by myself as well as by SOBELS. All these types are indicated in the experiments as type III.

³⁾ It is remarkable that, in my observations, only the animal blastomeres lose contact.

d. Third cleavage. The third cleavage of normal 4-cell stages can show several abnormalities. The normal dextrotropic cleavage-angle of 45° can be reduced, even to 0° . This is often accompanied with a reduction of the number of micromeres. Some other possibilities are shown by figs. 7 and 8.

e. Third cleavage in rotated 4-cell stages. Type I is capable of an entirely normal dextrotropic third cleavage. There is no evidence of a third cleavage of type II ⁴⁾. Type III, however, is capable of giving rise to rather regular, but also to very irregular 8-cell stages, a process illustrated by fig. 9. A more irregular 8-cell type is shown in fig. 10. These eggs often consist of less than 8 cells, never of more. Their most remarkable feature is the fact that nearly always all cells are situated in one plane. Possibly gravity causes a more or less horizontal position of the cleavage spindles by flattening the blastomeres of the 4-cell stage.

The four cell-divisions giving rise to an 8-cell type have been observed in a number of cases to occur perfectly simultaneously.

2. *Experiments*

Each batch (batches numbered 1—16) was divided into four lots of 5—10 eggs each (lots numbered *a—d*), which were transferred to the solution at different moments between laying and first cleavage.

For the lots five subjective degrees of retardation as compared with the control eggs ⁵⁾ were established: no, slight, moderate, rather strong and strong retardation. The last degree was left out of account with regard to rotation, as in those lots most of the eggs are arrested in development before reaching the second cleavage.

The rotation is given as the percentage of the total number of eggs of the lot. In this percentage types I, II and III are counted together.

The results do not lend themselves to statistical treatment. They can only point to possibilities, principally because the number of figures is too small.

a. Retardation of development. In general it is observed that the degree of retardation in lots of the same batch decreases as the lots have been placed into the solution at a later moment. Two batches show the opposite behaviour. Different batches show great variations in susceptibility, as far as retardation is concerned.

b. Rotation during second cleavage. With regard to rotation we also find great variations in susceptibility. Seven out of sixteen batches used do not show any rotation at all. They will be altogether left out of account.

If rotation occurs, the percentages in the lots of one batch do not differ very much, no matter what the degrees of retardation of the different lots are. The percentages in different batches may, however, show considerable differences. The following figures may serve as an example (Table I).

⁴⁾ Even if such a cleavage occurred it would be very difficult to interpret.

⁵⁾ The exact point of time of a cleavage in a certain lot was taken to be the moment at which 50 % of the eggs had cleaved.

TABLE I

Degree of retardation (* = no, ** = slight, *** = moderate, **** = rather strong) and percentage of eggs showing rotation of second cleavage spindles in different lots (*a, b, c, d*) of 4 batches (3, 9, 10, 15).

Batches	Lots			
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
3	**** 10 %	—	** 10 %	—
9	—	**** 10 %	**** 10 %	** 20 %
10	*** 30 %	*** 50 %	*** 40 %	—
15	** 75 %	** 60 %	** 75 %	—

To find an answer to the question, whether spindle rotation is always coupled with retardation of development, we have been looking for cases in which rotation occurred in an individual egg which did not show any delay of cleavage as compared with the controls, and which belonged to a lot which was not delayed as a whole either. Only in that case we can be sure that it does not concern an egg which is one of the first to cleave in its lot, and in control solution would have cleaved some time before the average time of cleavage of the controls. The following cases may be considered (Table II).

TABLE II

Degree of cleavage delay (cf. tab. I) in individual eggs showing rotation of second cleavage spindle

Lot	Degree of retardation of the lot	Rotation type	Indiv. delay of 2d cleavage	
2a	**	II	< 10 min.	no. 8 to cleave of lot of 10
9d	**	II	0 min.	no. 4 to cleave of lot of 10
15a	**	II	0 min.	no. 2 to cleave of lot of 8
15b	**	III	< 5 min.	no. 1 to cleave of lot of 5
15c	**	III	< 10 min.	no. 4 to cleave of lot of 8
16d	*	II	< 10 min.	no. 4 to cleave of lot of 8

Concerning the question whether spindle rotation occurs simultaneously with the changes in the direction of the spindles in the control eggs, it must be remarked that a second cleavage leading to a rotation type was never observed to appear at the same time as the third cleavage of the controls. Rotation type second cleavages may appear *at any time between* the second and the third cleavages of the controls, but most frequently about 10 to 60 minutes after the second cleavage, i.e. 75—135 minutes before the third cleavage of the controls.

c. Rotation during third cleavage. We only consider the types in which the normal cleavage-angle has been reduced to 0°. These types only occur in lots with no, slight or moderate retardation. Out of about 10 third cleavages observed in lots with rather strong or strong retardation, no one

was of this type, though most of them were either abnormal or showed smaller rotation-angles. Of 4 of the types "third cleavage 0" it is certain that they were not delayed in comparison with the controls, nor were the lots to which they belonged.

B. Sucrose

In order to investigate whether osmotic influences play a part in the induction of spindle rotation, a series of parallel experiments were carried out, in which, besides a 0.75 % solution of thiourea, a sucrose solution of the same osmotic pressure, i.e. of 3.3 %, was used, together with some other solutions of lower concentrations.

1. *Retardation of development*

In 3.3 % sucrose, retardation nearly always was stronger than in 0.75 % thiourea. The degree of retardation always was "strong". In lower concentrations of sucrose, i.e. from 3 % downwards, retardation nearly always was less than in 0.75 % thiourea. Mostly, and in any case from 2.5 % downwards, there was no retardation at all.

2. *Abnormalities and rotations*

It is remarkable that in 3.3 % sucrose solution the eggs behaved much more like those in hypertonic solutions (cf. GRASVELD 1949) than they did in 0.75 % solution of thiourea. The blastomeres were more rounded, and often lost contact especially after the 3rd cleavage. Sometimes a pointed shape of the first polar body was observed.

All three rotation-types occur in sucrose solutions of 2.4 % to 3.3 %, that is at all degrees of retardation. On two occasions the formation was observed of a radially symmetrical 3-cell type, possibly the result of the formation of a triaster during karyokinesis.

All three eggs of type II observed later showed a retrogression to a type in which the pairs of blastomeres formed an angle smaller than 90°. This phenomenon may be connected with the loose mutual attachment of the blastomeres.

C. Cold treatment

The eggs were kept at temperatures of -2° to $+6^{\circ}$ C, during periods varying from 6 to 36 hours. No spindle rotation could be induced in this way, the only result being a total arrest of development during treatment, and a retardation of development afterwards. Very much inhibited eggs showed a considerable increase in volume. The cleavage cavity always was larger than normally.

Discussion:

The most important fact shown by this investigation is that spindle rotation is not an effect which is specific for thiourea, but that it can also

be produced by sucrose. This leaves open the possibility that spindle rotation is an osmotic effect. In contradiction with CONKLIN's results in *Crepidula*, cold treatment failed to induce spindle rotation in *Limnaea*.

Of the two questions put in the introduction to verify SOBELS' explanation of spindle rotation, the second one can be answered in the negative. In 6 out of 8 batches concerned spindle rotation types appeared 75 minutes or more before the third cleavage of the controls.

The answer to the first question also seems to be negative, according to the data given in table II.

Other considerations speaking against SOBELS' explanation are:

Firstly: No rotation-types have been observed by me to originate from intermediate 3-cell stages, as described by SOBELS.

Secondly: The 8-cell types originating from rotation-type III remind us very much of a 3rd cleavage, especially as the four cell-divisions have been observed to occur simultaneously. According to SOBELS' explanation one would expect a transitory 6-cell stage, representing a "half, premature, fourth cleavage", soon followed by further cleavages.

Thirdly: The synchronicity of cleavage divisions can be disturbed without spindle rotation. This has also been observed by SOBELS himself.

Lastly: Disorientations of the cleavage spindles are not restricted to rotations in a plane parallel to the original first cleavage plane. This has been observed by SOBELS as well as by me (cf. fig. 5).

We must take into account the possibility that the more pronounced rounding off and the resulting looser mutual attachment of the blastomeres in thiourea and sucrose plays a part in the formation of rotation-types. In those cases, however, where the animal cell of a rotated pair is smaller than the vegetative one, we can be sure that a spindle rotation has taken place: the difference in cell size can easily be explained by the fact that the nucleus of a $\frac{1}{2}$ -blastomere is situated nearer the animal side of the cell. The direction of the spindle is the primary factor, the formation of a "micromere" is the effect ⁶⁾.

In the type of fig. 8 we also see that the meridional cleavage divisions result in pairs of blastomeres of equal size, whereas the "equatorial" cleavage divisions result in pairs of blastomeres of unequal size.

Summary:

1. Eggs of *Limnaea stagnalis* were cultured in a solution of thiourea of 0.75 % and in sucrose solutions varying between 2 % and 3.3 %.
2. In thiourea as well as in sucrose solutions retardation of development occurs, the degree of which decreases as the eggs are transferred to the solution at a later moment.
3. Rotation of second cleavage spindles occurs in thiourea as well as

⁶⁾ This of course applies to all cases of so-called "premature micromere formation".

in sucrose, and involves either one or both blastomeres. The third cleavage in thiourea may show an alteration in the normal cleavage angle of 45° .

4. Treatment with low temperatures failed to induce spindle rotation.

5. Spindle rotation seems not always to be coupled with retardation of development. Moreover in most cases spindle rotation during second cleavage occurs 75 minutes or more before the third cleavage of the controls. It is, therefore, not likely that spindle rotation in *Limnaea* can be compared with the so-called "premature micromere formation", observed in *Paracentrotus* and *Crepidula*.

6. Thiourea has a disorienting effect on the directions of the cleavage divisions and the situations of the cleavage planes in second and third cleavage. Spindle rotations during second cleavage are not restricted to planes parallel to the first cleavage plane.

7. In the cases where rotations occur, their number seems not to depend on the degree of retardation, but only on the nature of the batch to which they belong. Both as regards retardation and spindle rotation the batches show great individual differences in susceptibility.

In am much indebted to Prof. CHR. P. RAVEN for suggesting this investigation, for his continuous interest in the work and his valuable advice in the preparation of this paper.

I also wish to thank Dr P. D. NIEUWKOOP for his valuable and inspiring assistance.

L I T E R A T U R E

- CONKLIN, E. G., Proc. Amer. Philos. Soc. **79**, 179 (1938).
 GRASVELD, M. S., Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam **52**, 284 (1949).
 HÖRSTADIUS, S., Acta Zool. **9**, 1 (1928).
 HUDIG, O., Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam **49**, 554 (1946).
 RAVEN, CHR. P. and L. H. BRETSCHNEIDER, Arch. néerl. Zool. **6**, 255 (1942).
 RAVEN, CHR. P., Arch. néerl. Zool. **7**, 353 (1946).
 SOBELS, F. H., Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam **51**, 900 (1948).