

# The Gastrulation of the Partial Embryos of *Sphaerechinus*.

By

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Owing to the differences in the results obtained by DRIESCH and myself in regard to the number of cells utilized in the development of the partial larvae of the sea-urchin I re-examined the question last year<sup>1)</sup> using *Toxopneustes variegatus* for the purpose. My results showed that the partial blastulae that gastrulate at the same time as, or very soon after, the normal use a proportionate number of cells in the formation of the archenteron; while those gastrulating later may use a larger number. This result seemed to account for the difference between DRIESCH and myself, since he appears to have observed early gastrulae, while I had later ones. Having an opportunity during the present summer, while holding the Smithsonian Table at the Naples Station, to re-examine this question with *Sphaerechinus*, I did so, in order to see if in this form also, the one I had used in 1895, the early gastrula uses a proportionate number of cells, and later ones a larger number.

The eggs were fertilized in sea water, and their membranes shaken off immediately. They were then placed in HERBST's calcium-free solution, where they remained until segmentation began. At the two- and four-cell stages, eggs were taken out and again shaken until many of the blastomeres were separated. They were then put into sea water, where they continued to develop. The embryos were preserved at intervals, after gastrulation had begun, stained, mounted, and their cells (nuclei) counted.

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<sup>1)</sup> Archiv f. Entwicklungsmech. XIII. 1901.

In the first set some of the whole embryos had just finished gastrulating at 12.30 P. M., while many were not more than half completed. At 1.30 P. M. two  $\frac{1}{2}$  gastrulae (measuring  $15 \times 15$  divisions<sup>1)</sup> each) had in one case 450 cells in the wall and 53 in the archenteron, and in the other case (the cells in the wall were not counted) 63 cells in the archenteron. At this time a  $\frac{1}{4}$  gastrula (measuring  $11 \times 11$  divisions) had 227 cells in the wall and about 21 in the archenteron.

At 2.30 P. M. the following  $\frac{1}{2}$  blastulae and gastrulae were examined (Table I). The whole gastrulae that had completed gastrulation at this time had about 90 to 100 cells in the archenteron.

Table I. 2.30 P. M.

 $\frac{1}{2}$  Gastrulae.

Size	Stage	Cells in Wall	Cells in Archent.
$18 \times 18$	Blastula	550	
$16 \times 16$	-	425	
$17 \times 17$	-	450	
$16 \times 16$	Begin Gastr.	358 +	
$17 \times 15$	Gastrula not finished	475	33
$17 \times 15$	Gastrula nearly finish.		31

Table II. 2.30 P. M.

 $\frac{1}{4}$  Gastrulae.

Size	Stage	Cells in Wall	Cells in Archent.
$13 \times 12$	Gastrula not finished	253	12
$12 \times 12$	Gastrula excentric	215	40
$13 \times 12$	Gastrula excentric	250	40

At 3.30 P. M. one  $\frac{1}{2}$  gastrula (measuring  $17 \times 16 +$ ) had 38 cells in the archenteron. Two  $\frac{1}{4}$  gastrulae (measuring  $12 \times 12$  and  $11 \times 10$ ) had in one case 188 cells in the wall and the beginning of an archenteron (not possible to count the cells), and in the other case 60 cells in the archenteron. In the last case the number appears proportionately too large, although the invagination appeared not to be completed. The archenteron is also proportionately too large.

A 4.30 P. M. a  $\frac{1}{2}$  blastula ( $17 \times 17$ ) which showed no signs of gastrulating had 580 cells in its wall, another ( $18 \times 18$ ) beginning to gastrulate (20 cells turned in) had 510 cells in the wall. A  $\frac{1}{2}$  gastrula (not completed) had 470 cells in the wall, and 52 in the archenteron; another ( $18 \times 14$ ) had 468 cells in the wall and 85 in the archenteron; and another ( $18 \times 16$ ) had 55 cells in the archen-

<sup>1)</sup> In this paper 18 divisions equal  $\frac{1}{10}$  mm.

teron. At this time most of the  $\frac{1}{2}$  larvae appeared to have about half-gastrulated.

At the same time (4.30 P. M.) three  $\frac{1}{4}$  gastrulae gave the following: One ( $12 \times 10$ ), only half-gastrulated, had 195 cells in the wall and 49 in the archenteron. The archenteron was disproportionately broad and large. Another ( $12 \times 11$ ) had 200 cells in the wall and 25 in the archenteron, which had just begun to turn in. A third ( $12 \times 12$ ) had 214 cells in the wall, and had just begun to invaginate.

At 5.30 P. M. the whole gastrulae ( $26 \times 23$ ) had about 125 cells in the archenteron. The two following tables give the results for  $\frac{1}{2}$  and  $\frac{1}{4}$  gastrulae at this time.

Table III. 5.30 P. M.

 $\frac{1}{2}$  Gastrulae.

Size	Stage	Wall	Archent.
$19 \times 17\frac{1}{2}$	Gastrula	—	50
$18\frac{1}{2} \times 16$	-	—	40
$18 \times 18$	Gastrula not finished	—	33
$16 \times 15$	Gastrula	440	88
$16 \times 14\frac{1}{2}$	-	—	56
$16 \times 15$	-	—	67
$16 \times 15$	Half Gastr.	—	60
$15 \times 15$	Gastrula	560	75
$19 \times 16$	—	475	80

Table IV. 5.30 P. M.

 $\frac{1}{4}$  Gastrulae.

Size	Stage	Wall	Archent.
$14 \times 14$	Gastr. begun	214	
$12 \times 12$	Blastula	250	
$13 \times 12\frac{1}{2}$	-	325	
$12 \times 12$	Gastrula excentric	185	25
$14 \times 12$	Gastrula	200	26
$13 \times 11\frac{1}{2}$	-	250	31
$13 \times 13$	Gastr. begun		23
$13 \times 12$	$\frac{2}{3}$ Gastrula		28
$12 \times 12$	$\frac{1}{2}$ Gastrula		29
$10 \times 10$	Gastrula	223	28
$13 \times 11$	-	175	48
$11 \times 9$	$\frac{1}{4}$ Gastrula	146	33

In Table III it is seen that the number of cells of the archenteron is larger than half of the whole number, although the number in the wall is not more than half. In Table IV a number of  $\frac{1}{4}$  gastrulae have disproportionately too many archenteric cells, and some of those in which the invagination is not even completed have a few more than a fourth of the whole number.

The results of the second series of observations are given in the following tables, etc.: At 9.30 A. M. the whole blastulae ( $22 \times 22$ ) had about 950 cells in the wall. Two  $\frac{1}{2}$  blastulae had in the one case ( $15 \times 15$ ) 480 cells in the wall and in the other ( $14 \times 14$ ) 433 cells,

i. e., almost exactly half the whole number. At 11.30 one whole blastula ( $23 \times 23$ ) had about 1000 cells in the wall, and a whole gastrula had about 940 cells in the wall and about 100 in the archenteron. At this time a few whole forms had completed gastrulation. A few of the  $\frac{1}{2}$  blastulae had begun to invaginate, and I even found three  $\frac{1}{4}$  blastulae which were also gastrulating. One of these ( $10 \times 10$ ) had 200 cells in the wall and the other ( $11\frac{1}{2} \times 11\frac{1}{2}$ ) 180 cells. One that had about finished gastrulating ( $11\frac{1}{2} \times 11\frac{1}{2}$ ) had 23 cells in the archenteron and about 215 cells in the wall.

At 4.45 P. M. the whole gastrulae swimming at the top had about 1000 cells in the wall, and those that had completed gastrulation had turned in between 90 and 110 cells. Those on the bottom were not quite so far advanced. They had from 800 to 900 cells in the wall, and one that had not completed gastrulation had 50 cells in the archenteron. One  $\frac{1}{2}$  gastrula ( $17 \times 17$ ) from the top had 304 cells in the wall and about 30 or more in the archenteron. A  $\frac{1}{2}$  gastrula ( $19 \times 17$ ), from the bottom, had 355 cells in the wall and 40 in the archenteron. A  $\frac{1}{4}$  gastrula ( $12 \times 8$ ) had 230 cells in the wall, and 16 in the incomplete archenteron; another ( $13 \times 13$ ), from the bottom, had 255 cells in the wall and 25 in the incomplete (?) and very oblique archenteron.

Two hours later, 6.30 P. M., three  $\frac{1}{2}$  gastrulae from the top (the first three in the table) and two from the bottom gave the following results: —

Table V. 6.30 P. M.  $\frac{1}{2}$  Gastrulae.

Size	Stage	Wall	Archent.
$19 \times 19$	Gastrula	500	50
$20 \times 20$	-	500	45
$20 \times 20$	-	—	55
$19 \times 19$	-	—	40
$19 \times 19$	-	—	30

At this time the whole gastrulae ( $28 \times 28$ ) had about 100 cells in the archenteron. Two  $\frac{1}{4}$  gastrulae had, in one case ( $14 \times 14$ ) 260 cells in the wall and 45 in the archenteron, and in the other case ( $15 \times 14$ ) 30 cells in the archenteron.

It will be seen that while the  $\frac{1}{2}$  gastrulae have about the proportionate number, one, of the  $\frac{1}{4}$  gastrulae has proportionately too many.

At 9.30 P. M. the whole gastrulae ( $28 \times 28$ ) had about 125 cells in the archenteron. Three of the  $\frac{1}{2}$  gastrulae from the top ( $21 \times 21$ ,  $23 \times 20$ ,  $20 \times 20$ ) had respectively about 68, 75 and 61 cells in the archenteron. One  $\frac{1}{4}$  gastrula ( $15 \times 15$ ) had 240 cells in the wall and 30 in the archenteron, which in this case did not appear disproportionately large, but lay somewhat excentrically. From the bottom the following  $\frac{1}{2}$  and  $\frac{1}{4}$  gastrulae were examined.

Table VI. 9.30 P. M.

 $\frac{1}{2}$  Gastrulae.

Size	Wall	Archent.
$20 \times 18$	400	60
$19 \times 16$	415	46 (small)
$19 \times 16$	400	67

Table VII. 9.30 P. M.

 $\frac{1}{4}$  Gastrulae.

Size	Wall	Archent.
$14 \times 12$	240	30
$13 \times 13$	—	25
$15 \times 14$	230	35
$13 \times 13$	240	23
$14 \times 13$	235	25

## General Conclusions.

The principal result is here the same as that which I looked upon as the main one in my first experiments; viz., that there is in the partial larvae no regulation of the cell-size. The cells in the  $\frac{1}{2}$  larva are proportionately twice too big, as compared with those of the whole larva, and in the  $\frac{1}{4}$  larva four times too big. The early gastrulae of Sphaerechinus, as in Toxopneustes, turn in, to form the archenteron, a proportionate number of cells. I found again, as I had observed in Toxopneustes, that in a large number of these gastrulae the archenteron is quite excentric. The early whole gastrulae of Sphaerechinus and Toxopneustes are practically radially symmetrical. Hence I can only interpret the excentricity of the archenteron of the partial larva as due to an incomplete regulation; a trace of the former structure still showing itself in the archenteron of the partial larvae. DRIESCH's criticism<sup>1)</sup> in regard to this point, based on an examination of Echinus, does not touch the problem, for, as the archenteron of the whole larva of this form is in an excentric position there are no means of determining to what the excentricity of the archenteron of the partial larva is due. Moreover

<sup>1)</sup> Archiv f. Entwicklungsmech. XIV. 1902.

the excentricity of the partial larvae of *Toxopneustes* is much greater than that in *Echinus*.

I have noticed again, as I had done before both for *Sphaerechinus* and for *Toxopneustes*, the disproportionately large size of the archenteron in the  $\frac{1}{4}$  forms. In part this may be due to the fact that the small blastulae do not enlarge in the same proportion as do the whole, but as DRIESCH has shown, in proportion to their surfaces, not to their volumes; yet I do not believe this gives a satisfactory explanation of the disparity in size of the archenteron in many of these partial larvae.

It is noticeable, especially in the later gastrulae (see Table IV), that the archenteron, when only half invaginated, may contain as many as, or even more than, one-fourth of the whole number of cells. It was this fact that I described in my first paper, and it was one of the main reasons that led me to conclude that the partial larvae tend to make use of the typical number of cells in forming the archenteron. The explanation that I gave in my last paper is, I believe, more satisfactory; viz., that in the partial blastulae, in which the invagination is retarded, the cells in the archenteric plate slowly increase in number (as do the cells in the archenteron of the whole gastrula after invagination), so that a disproportionately large number may appear even before the gastrulation is completed. One should be very careful to distinguish between these incomplete gastrulae and those that are complete but turned to one side. By rotating the larvae it is easy to tell which of these conditions is present. It is also noticeable in some of the late partial larvae that a proportionately larger area is involved in the inturning of the archenteron, and this is apparently connected with the disproportionate size of the archenteron.

### **The Number of Cells Invaginated by *Strongylocentrotus*.**

The recent interesting results<sup>1)</sup> obtained by BOVERI in regard to the orientation and gastrulation of *Strongylocentrotus* led him to the conclusion that half of the cells of the blastula-wall is turned in to form the mesenchyme and the archenteron. If this conclusion is correct then *Strongylocentrotus* differs from the other sea-urchins

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<sup>1)</sup> Verhandl. phys.-med. Ges. Würzburg. XXXIV. 1901; und Zool. Jahrb. XIV. 1901.

that I have examined, viz., Sphaerechinus, Echinus, and Toxopneustes, which turn in only about one-tenth of the total number of cells in the blastula. BOVERI has possibly overestimated the extent of the inturning of cells in Strongylocentrotus. The question has another bearing of some theoretical interest. If half of the blastula wall becomes endoderm, then DRIESCH's results from cutting the blastula in two may have a different interpretation, for it would be difficult to obtain a piece of the anterior part of the blastula which does not contain some endodermal cells. If it contained such cells we might not be justified in concluding that every region of the blastula has the power of forming endoderm.

I examined, therefore, with some interest the process of invagination of Strongylocentrotus, and found the same rule holds here that I had made out for the other species. About one-tenth of the whole number of cells is invaginated, and not one-half as BOVERI's conclusion demands.

Thus I found about 800 cells in the blastula wall an hour and a half (at 9.30 A. M.) before gastrulation begins. At 11 A. M. gastrulation had just been completed in a few individuals, and in one of these I counted about 800 cells in the wall and about 100 in the archenteron. Again at 4 P. M. there were, in one count, about 900 cells in the wall and 135 in the archenteron. I conclude, therefore, that BOVERI's estimate is much too high, for, if it were correct, nearly 400 cells should be turned in, while in reality only about 100, or  $\frac{1}{9}$  the total number are invaginated. DRIESCH<sup>1)</sup> has also called attention to this discrepancy, in an article that has just reached me. He gives also the number of cells of the mesenchyme in Strongylocentrotus. He estimates that 50 cells wander in to form this tissue, and if we add this number to the 100 cells of the archenteron, we see that the total number is still far below that of the estimate derived from BOVERI's results.

### Summary.

1) The  $\frac{1}{2}$  and  $\frac{1}{4}$  larvae of Sphaerechinus contain only a half and a fourth respectively of the total number of cells in the whole larva. These cells are, therefore, proportionately twice and four times too large. There is no regulation in cell-size.

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<sup>1)</sup> Archiv f. Entwicklungsmech. XIV. 1902.

2) The  $\frac{1}{2}$  and  $\frac{1}{4}$  blastulae that gastrulate at the same time as, or soon after the whole blastulae, use a proportionate number of cells for the archenteron, viz.: one-tenth of the whole number.

3) The archenteron, especially of the early gastrulae, is often very excentric, which is probably due to an incomplete regulation; showing that a trace of the original structure is still present.

4) The partial blastulae that gastrulate later turn in proportionately more cells than one-tenth of the whole number, as in Toxopneustes. When only half-gastrulated they sometimes have more than the proportionate number of cells in the archenteron.

5) A larger area of the archenteric plate is involved in the late partial larvae, and the archenteron, especially in the  $\frac{1}{4}$  gastrulae, is often disproportionately too large.

6) Strongylocentrotus appears to be governed by the rule followed by other sea-urchins, and invaginates about one-tenth of the whole number of cells, and not one-half as BOVERI's results seem to indicate.

### Zusammenfassung.

1) Die ganzen Halbei- und Viertel-Larven von Sphaerechinus enthalten nur die Hälfte und bezw. ein Viertel der Totalanzahl von Zellen in den Ganzei-Larven. Diese Zellen sind daher, in entsprechendem Verhältnis, zwei- und viermal zu groß. Eine Regulation der Zellgröße giebt es dabei nicht.

2) Die Halbei- und Viertel-Blastulae, welche gleichzeitig oder bald nach den Ganzei-Blastulae die Gastrula bilden, verwenden eine verhältnismäßig entsprechende Anzahl von Zellen für den Urdarm, nämlich ein Zehntel der Gesamtzahl.

3) Der Urdarm ist oft, ganz besonders in den frühzeitig gebildeten Gastrulae, sehr excentrisch, was wahrscheinlich auf einer unvollständigen Regulation beruht; es zeigt dies, dass noch eine Erinnerung an die eigentlich normalen Bauverhältnisse besteht.

4) Die später gastrulirenden Eitheil-Gastrulae stülpen verhältnismäßig mehr Zellen ein, als ein Zehntel der Gesamtzahl, wie bei Toxopneustes. Wenn die Gastrulation erst halb vollendet ist, haben sie manchmal mehr als die verhältnismäßige Zellenzahl im Urdarm.

5) Ein noch größeres Feld der Urdarmplatte wird in den späten Theilarven eingestülpt, und der Urdarm ist oft, speciell bei den Viertel-Gastrulae, zu groß.

6) Für Strongylocentrotus scheinen dieselben Regeln zu gelten, denen die anderen Seeigelarten folgen. Er stülpt etwa ein Zehntel der gesamten Zellenzahl ein, und nicht die Hälfte, wie BOVERI's Ergebnisse anzudeuten scheinen.