

II.—*The Ascidian Half-Embryo.*

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The development of isolated blastomeres of the ascidian egg has afforded a subject of considerable discussion on the part of many theoretical embryologists. Chabry* approached the subject from the experimental side, and, from the results of his many detailed observations and experiments, was led to the conclusion that one of the isolated blastomeres of the two-celled stage produced a strict half-embryo. As it was well known that the first cleavage-plane divided the egg into right and left halves, this conclusion seemed altogether probable and of considerable interest.

A number of writers, however, among them Hertwig,† Driesch,‡ Weismann,§ Barfurth|| and Roux,¶ were led, on the grounds of Chabry's results, to opinions more or less at variance with his. Barfurth considered Chabry to be in greater part correct. Roux and Weismann believed that during the later development the missing part was supplied by the other cells through "postgeneration." Hertwig states that, in his opinion, Chabry was in error; and Driesch also argued that a typical total development occurred. Finally, Driesch** in 1893 repeated Chabry's experiments, upon the eggs of *Phallusia mammillata*, and by the results wholly confirmed the theoretical conclusions of his previous paper.

* Chabry L. Contribution à l'embryologie normale et tératologique des ascidies simples. Journ. de l'anat. et de la phys. XXIII. 1887.

† Hertwig, R. Urmund und Spina bifida. Arch. f. mikr. Anat. XXXIX. 1892.

‡ Driesch, H. Der Werth der beiden ersten Furchungszellen in der Echinodermmentwicklung. Zeit. f. wiss. Zool. LIII.

§ Weismann, A. Das Keimplasma. 1882.

|| Barfurth, D. Halbbildung oder Ganzbildung von halber Grösse. Anat. Anz. VIII. 1893.

¶ Roux, W. Über des entwickelungsmechanische Vermögen jeder der beiden ersten Furchungszellen des Eies. Verhandl. d. Anat. Ges. Wien. 1892.

** Driesch, H. Von der Entwicklung einzelner Ascidienblastomeren. Archiv für Entwick. der Organismen. I. 3. 1895.

Although at that time reluctant to admit anywhere the occurrence of "partial" development, Driesch has since proved, in connection with Morgan, the existence of a partial *early* development in the ctenophore egg.* And in a recent paper by the writer† it has been shown that the isolated blastomere of the snail possesses the power of forming only a corresponding portion of an embryo. In a later paper, Driesch,‡ developing an idea suggested by Prof. E. B. Wilson and myself (loc. cit.), recognized the existence of a series among animal eggs, from the nearly isotropic eggs of the medusa, Amphioxus, fish, sea-urchin, etc., at one extreme, to forms such as the frog and ctenophore, and finally to the snail, at the other extreme, where the blastomere possesses such an organization that but a part of an embryo can be formed and postgeneration cannot occur.

The ascidian egg, however, remained unexplained by the contradictory results of Chabry and Driesch. From this consideration the author was led to an examination of the facts in another ascidian. The results will, it is hoped, clear up the confusion to some extent, and will show how far the development is a "partial" one and in what respects it is "total."

The experiments were performed during the past summer at the Marine Biological Laboratory, Wood's Holl, upon the eggs of *Molgula manhattensis*, which grows very abundantly upon the piles and wharves at New Bedford, Mass. Artificial cross-fertilization was resorted to, and the eggs at the desired stage were spurted in a watch-glass by means of a fine spiral pipette.§ Those eggs presenting isolations were placed separately in watch-glasses, and camera drawings of successive stages were made at intervals, using a Zeiss oc. 4, and obj. C.

As to nomenclature, the system proposed by Kofoed|| and ap-

* Driesch, H., and Morgan T. H. Von der Entwicklung einzelner Ctenophorenblastomeren. Archiv für Entwick. der Organismen. II. 2. 1895.

† Crampton, H. E., Jr. Experimental Studies on Gasteropod Development, with an appendix on Cleavage and Mosaic Work, by E. B. Wilson. Archiv für Entwick. der Organismen. III. 1. 1896.

‡ Driesch, H. Betrachtung über die Organisation des Eies und ihre Genese. Archiv für Entwick. der Organismen. IV. 1. 1896.

§ As previously described in connection with the gasteropod experiments.

|| Kofoed, C. On some laws of Cleavage in Simax. Proc. Amer. Acad. Arts and Sciences. Vol. XXIX. 1894.

plied by Castle* to the *Ciona* egg has been used for obvious reasons. According to this system, now well known, each cell is designated by a letter referring to the particular quadrant of the four-cell stage from which it arose; in addition it receives an exponent denoting the generation to which it belongs, and a second exponent denoting its place in that generation, counting from below upward.

DETAILED DESCRIPTION OF CLEAVAGE.

A. *Normal Cleavage*.—The cleavage of the *Molgula* egg is precisely the same as that of *Ciona* and other ascidians, as far as it has been followed. Therefore, it is unnecessary to discuss the normal phenomena further than to emphasize a few of the facts which are important in connection with the cleavage of the fragments.

The first and second cleavage-planes are meridional, while the third is equatorial. An eight-cell stage results (fig. 1) which, seen from the side, consists of two tiers of four cells each. The upper tier is shifted anteriorly upon the lower, so that the *posterior upper* cells are in contact with the *anterior ventral* cells. This relation is constant, and characteristic of probably all ascidian eggs (Castle, loc. cit., p. 228). Passing to the 16-cell stage, all the eight blastomeres divide. The spindle axes are inclined in such a manner that the *anterior* products of the *anterior* cells (fig. 2: B⁵⁻², b⁵⁻⁴) lie slightly *below* the median products; while the *posterior* products of the *posterior* cells lie slightly *above* the other cells (fig. 2: C⁵⁻¹, c⁵⁻³). When activity is again resumed, the dorsal cells remain quiescent, while the ventral cells segment, and a 24-cell stage results (fig. 3). After a period of rest the dorsal cells pass into the same generation (sixth) with the ventral cells, and a morula of 32-cells results. Then the ventral cells divide at about the same time, while the dorsal cells remain quiescent, giving a 48-cell stage.

Further details are unnecessary for our purpose. We emphasize the fact that, beginning with the 16-cell stage, there is a well-marked alternation of activity between the cells of the upper and those of the lower hemisphere of the embryo.

* Castle, W. E. The Early Embryology of *Ciona intestinalis*. Bulletin of Mus. of Comp. Zool. Harvard. Jan. 1896.

B. *Cleavage of the $\frac{1}{2}$ blastomere*.—As is well known, the isolation of an ascidian blastomere is effected by the death of its neighboring cell or cells, and not by an actual separation. The dead cell partially disintegrates and exerts upon the living cell no modifying influence, such as mechanical obstruction to rounding during division, etc.

$\frac{2}{4}$. At the normal time, viz: at the time of division of control eggs, the injured blastomere divides about equally (figs. 4 and 13). Often when the eggs are operated upon when passing into the 4-cell stage, evidence of division in the dead cell will remain. In such cases the division plane of the living cell is seen to be meridional and at right angles to the first. Therefore, it corresponds with the second cleavage-plane of the normal embryo. In all cases where it is possible to ascertain the facts this relation obtains. Driesch finds in *Phallusia* that no such constancy of relation exists.

$\frac{4}{8}$. After a normal period of rest the two cells divide at the same time. There are thus produced four cells which are arranged in a manner exactly similar to the half of a normal 8-celled embryo. Seen from the side (figs. 5, 9) the cells lie so that two are separated, while two are in contact; these latter are the posterior dorsal and the anterior ventral cells, as shown by the succession of the cleavage planes of the fragment. Precisely as in the normal 8-celled embryo, there is an anterior shifting of the dorsal cells upon the lower cells. According as this shifting is to the right or left, in lateral view, one is confronted by a right or left half-embryo. From a comparison of the figures, it is seen that the embryo in fig. 5 is the same as the half turned toward the observer of fig. 1; while that shown in fig. 9 is derived from a right $\frac{1}{2}$ blastomere. The appearance of the $\frac{4}{8}$ embryo in end view is shown in fig. 14, and a characteristic crossing of the spindle axes is exhibited, which is similar to their crossing in the complete egg (vide Castle for figures). The four-celled fragment, then, is in nowise a counterpart of the normal four-celled embryo, but, on the contrary, corresponds in *every* particular to the half of an eight-celled embryo.

From Chabry's fig. 106, it appears that a typical $\frac{4}{8}$ stage occurs also in *Ascidicella*.

$\frac{8}{16}$. At the next cleavage, all the cells divide (figs. 6, 10). Exactly as in the origin of corresponding normal cells, (fig. 2)

the anterior products of the anterior cells (fig. 6: $B^{5.1}$, $b^{5.3}$; figs. 10 and 16: $A^{5.1}$, $a^{5.3}$) lie slightly below the other cells; and the posterior products of the posterior cells (fig. 6: $C^{5.2}$, $c^{5.4}$; figs. 10 and 16: $D^{5.2}$, $d^{5.4}$) lie slightly above the median products. On a comparison of fig. 6 and fig. 2, it will be seen, however, that the topographical relations of the cells of the fragment are quite different from the normal. For example in fig. 6, the cell $c^{5.4}$ is in contact with $B^{5.1}$ and $b^{5.3}$, while in the normal egg it lies at the other end of the embryo. A similar rearrangement is still better shown in fig. 10, that of a right $\frac{1}{6}$ embryo, where $D^{5.2}$ is in contact with $A^{5.1}$, while $d^{5.4}$ is in contact with $A^{5.1}$ and $a^{5.3}$. This rearrangement is obviously rendered possible by the absence of the other half of the embryo, so that the cells cohere in a spherical form just as a corresponding number of soap-bubbles. It cannot be considered as a "gliding," for the spindle-axes are from the first accommodated to the changed conditions. That is (figs. 15, 16), the anterior end of the anterior spindles, and the posterior ends of the posterior mitotic figures are swung somewhat toward the original first cleavage-plane of the embryo.

Chabry's fig. 113 leaves no doubt that the $\frac{1}{6}$ embryo of *Ascidella* is precisely the same as that described above for *Molgula*. From Driesch's fig. 5, there is no doubt that in *Phallusia* the eight cells are arranged as the normal 8 cells.

$\frac{8}{16} - \frac{1}{2}$. When activity is again resumed, only the four lower cells are affected, while the dorsal cells remain quiescent. A 12-celled fragment results (figs. 7 and 11), which is exactly equivalent to a half of the normal 24-cell stage (fig. 3). The quiescence of the dorsal cells during the division of the ventral cells is the first indication of the alternation of activity in the rhythm of cleavage, which was found to be characteristic of this type of segmentation. As in the preceding stage, when the resting condition is assumed, there is a passive rearrangement of the cells. For example, the cells $A^{6.3}$ and $A^{6.4}$ were segmented along an axis inclined at an angle of 45° to the axis joining their centres at the resting stage. Again the cells $D^{6.3}$ and $D^{6.4}$ have retreated around the posterior end of the fragment.

$\frac{1}{32}$. While the eight cells of the lower hemisphere are resting, the four dorsal cells likewise pass into the sixth generation, and a $\frac{1}{32}$ stage results (figs. 8, 12). Its resemblance to the half of a normal 32-cell stage is still less marked than that of a $\frac{1}{16}$ embryo

to a half of the normal 24-celled stage. This is so, for the reason that further passive rearrangements of the cells occur, obscuring the partial character of their origin, and causing the cell complex in its solid, or "complete," condition to resemble a normal or "complete" embryo. Nevertheless, the succession of rhythmic cleavages, relation of successive cleavage-planes, etc., point to the operation of factors which are counterparts of those operating in a half of the normal embryo.

Later development. The embryo is now "complete," and gives rise to a complete blastula and larva. Although the process of gastrulation has not been carefully observed, enough of the later development has been ascertained to prove that a larva arises which resembles the normal larva, except as regards its smaller size and certain minor defects. My results, therefore, are entirely confirmatory of those of Driesch upon *Phallusia*.

C.—Cleavage of the $\frac{1}{4}$ blastomeres.—One of the isolated blastomeres of the four-cell stage, is divided at the next cleavage by a plane which is seen to be at right angles to both of the preceding planes. Therefore it corresponds to the third cleavage plane of the normal embryo. The $\frac{2}{8}$ stage is shown in fig. 17. A subsequent cleavage cuts each of the cells equally, and a $\frac{4}{16}$ stage results (figs. 18, 19), until this time, one is left in doubt as to the true nature of the fragment, that is, whether it will segment as a quarter or as an entire egg. However, from this time on, the character of cleavage is exactly that of a quadrant of a normal embryo.

When division next occurs, only the two cells toward the observer segment (fig. 20), and a stage of six cells results, which is evidently comparable to a $\frac{6}{32}$ embryo only, and not to any stage of the normal development. After a normal period, the dorsal cells (lower in the figure) pass into the sixth generation, and an $\frac{8}{32}$ embryo (fig. 21) is the result. As in the previously described fragments, passive rearrangements occur when the resting condition is assumed, and the cells flatten down upon one another (fig. 22). The cells of the ventral half segment at the next period of activity (fig. 23), while the dorsal cells remain undivided. The resulting $\frac{1}{16}$ stage, although solid, is nevertheless derived from the $\frac{1}{4}$ blastomere through a segmentation of a partial character. This partial character is expressed chiefly in the characteristic rhythm of cleavage.

Concerning the later stages, the results of Driesch are again confirmed. The young larvæ represented in Figs. 25, 26 of this paper illustrate one point further, although of minor consequence. It will be seen that the long axis of the $\frac{1}{4}$ larva in fig. 25, and the long axes of the $\frac{1}{4}$ larvæ derived from the same egg, in fig. 26, are approximately parallel to the principal dorso-ventral-axis of the original egg.

SUMMARY AND CONCLUSION.

An isolated blastomere of the *Molgula* egg segments as if still forming a corresponding part of an entire embryo. The cleavage phenomena are strictly partial, as regards the origin of cells, the inclination of cleavage-planes, and especially in respect to the rhythm of segmentation. The general appearance of the fragment differs materially from that of a half of a complete embryo, for the reason that rearrangements of the blastomeres occur, which tend progressively to mask the partial nature of development. The end result is a larva of less than normal size, and with defects in certain of its systems. These defects are undoubtedly due to the fact that but a portion of the normal amount of material is available for the formation of the larva; that, for instance, the chorda of a larva derived from a one-half blastomere, receives but one-half of the normal number of cells, and consequently a chorda of one row, and not two rows of cells, results.

In conclusion, one is constrained to adopt the view of Roux—namely, that in *Molgula* as in the well-known case of the echino-derms (Driesch, Wilson, and others) the development begins as a partial one, but that the missing part is gradually supplied by the cells already present. Driesch is also entirely correct, as far as the end result, a nearly complete larva, is concerned.

EXPLANATION OF PLATE IV.

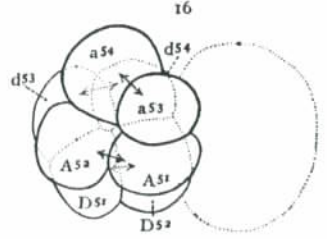
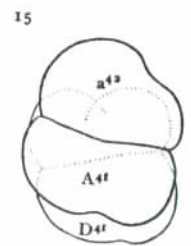
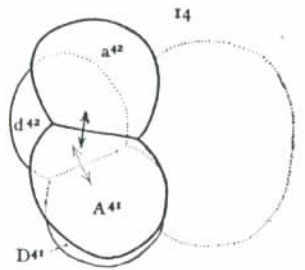
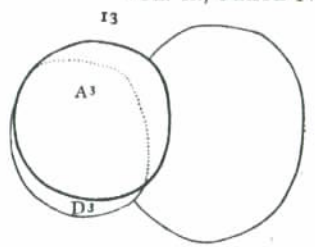
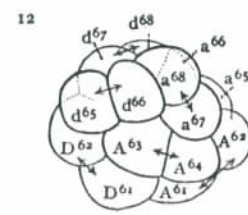
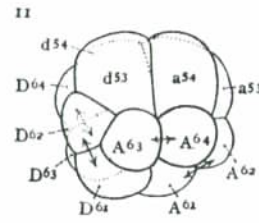
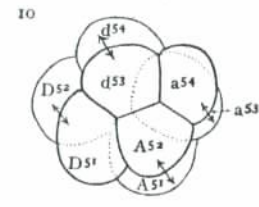
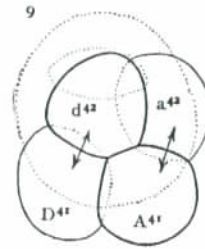
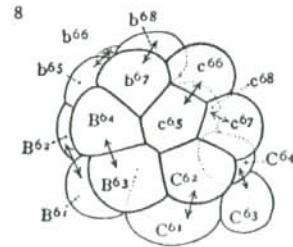
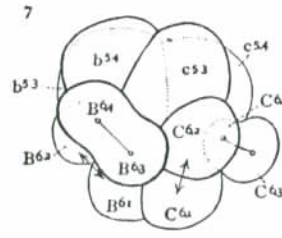
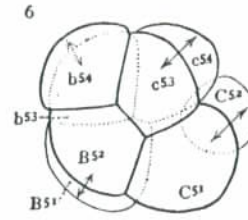
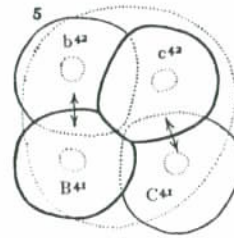
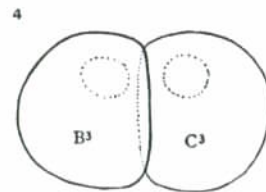
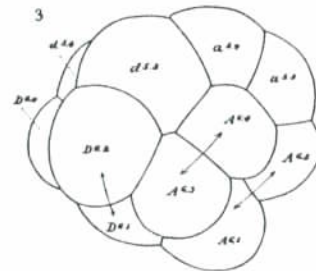
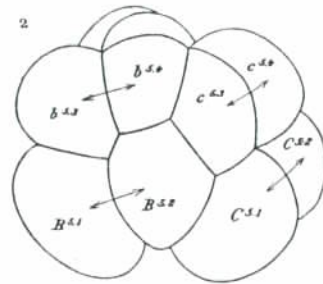
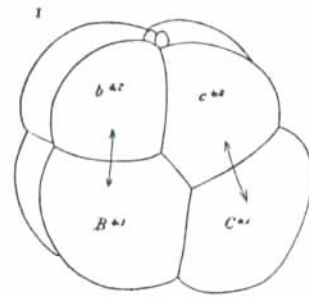
Magnification of figs. 1-3 about 280 diameters; of all other figures, 250 diameters. The arrows show the direction of cleavage.

- Fig. 1, 8-cell stage of *Ciona* from Castle (fig. 23), from the left side.
- Fig. 2, 16-cell stage of *Ciona* from Castle (fig. 24), from the left side.
- Fig. 3, 24-cell stage of *Ciona* from Castle (fig. 43), from the right side.
- Figs. 4-8, cleavage of the left $\frac{1}{2}$ blastomere of *Molgula*, from the side.
- Fig. 4, $\frac{1}{4}$ embryo.
- Fig. 5, $\frac{1}{4}$ embryo.

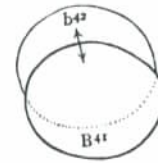
- Fig. 6, $\frac{1}{6}$ embryo.
- Fig. 7, passage to $\frac{1}{2}$ embryo.
- Fig. 8, $\frac{1}{2}$ embryo.
- Figs. 9-12, cleavage of the right $\frac{1}{2}$ blastomere, from the side.
- Fig. 9, $\frac{1}{4}$ embryo.
- Fig. 10, $\frac{1}{6}$ embryo.
- Fig. 11, $\frac{1}{2}$ embryo.
- Fig. 12, $\frac{1}{2}$ embryo.
- Figs. 13-16, cleavage of the right $\frac{1}{2}$ blastomere, from the front.
- Fig. 13, $\frac{1}{4}$ embryo.
- Fig. 14, $\frac{1}{4}$ embryo.
- Fig. 15, passage to $\frac{1}{6}$ embryo.
- Fig. 16, complete $\frac{1}{6}$ embryo.

EXPLANATION OF PLATE V.

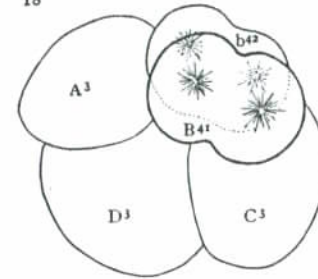
- Figs. 17-24, cleavage of the $\frac{1}{4}$ blastomere, ventral view.
- Fig. 17, $\frac{1}{4}$ embryo.
- Fig. 18, passage to $\frac{1}{6}$.
- Fig. 19, complete $\frac{1}{6}$.
- Fig. 20, $\frac{1}{4}$ embryo.
- Fig. 21, $\frac{1}{2}$ embryo, immediately after division.
- Fig. 22, $\frac{1}{2}$ embryo, in resting condition.
- Fig. 23, passage to $\frac{1}{4}$ stage.
- Fig. 24, complete $\frac{1}{4}$ embryo.
- Fig. 25, $\frac{1}{4}$ larva. The arrow indicates the long axis.
- Fig. 26, two $\frac{1}{4}$ larvæ, from same egg. The arrows indicate the principal axes.



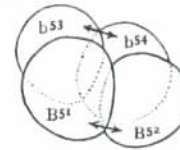
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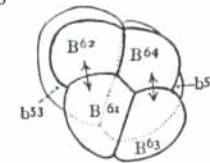
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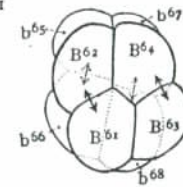
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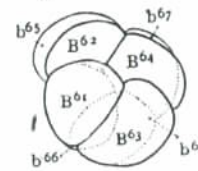
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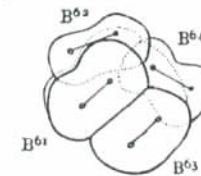
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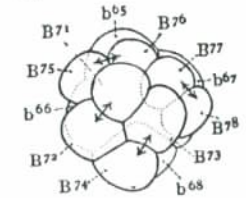
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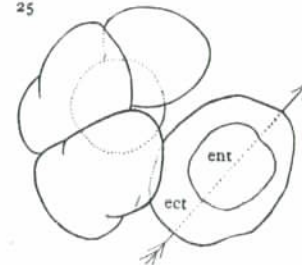
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