The Formation of one Embryo from two Blastulae.

By

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With plate IX.

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In an experiment of a series made to study the development of egg-fragments of Sphaerechinus I found spherical and dumb-bell shaped blastulae, which had double the volume of ordinary blastulae. Further examination showed many such blastulae fused to one another in varying degrees. Measurements of these double forms (22 hours old) showed unquestionably that each was made up of two blastulae more or less fused into one (Figs. 2—6). The conditions under which the experiment had been made were as follows. The eggs, two minutes after fertilization, had been shaken vigorously in a small tube. The membranes of the eggs had been thus removed as soon as formed. Some of the eggs had been broken up into fragments, but many others had not been broken by the shaking. The eggs after shaking had been dropped from the height of about a foot into a large dish of sea-water where they soon came to rest forming a thick layer on the bottom. In the one most successful experiment in which all of the eggs had come from the same female a great many double blastulae were found. At what stage the fusion had taken place I do not know[1]). I have repeated the experiment very many times generally without success, having only two other times gotten a similar result.

Once again, under the same conditions, a large number of fused blastulae were produced. In this experiment large numbers of blastulae, four, five, six and more appeared stuck to one another, but generally had separate segmentation cavities.

I varied the conditions of the experiment. For instance, the

[1]) Later experiments show that the fusion takes place at the blastula stage.

eggs were dropped from warmer into colder water and vice versa, they were put into deluted sea-water, and dropped from the tube into water from different heights but without result. It should be recorded that in the first experiment the sea-urchins had been kept for several days in an aquarium, but later when this was done again no double embryos were obtained from the eggs. Probably some peculiarities of the eggs of certain females are favorable for fusion, and the result did not depend entirely on external agencies.

I examined eggs, immediately after they had been treated by the method described above, and found in all cases that a certain number of unsegmented eggs without membranes were stuck together. They were found united in twos and threes as shown in Figs. 20 and 21. So closely were these eggs stuck to one another, by the inner clear coat, that each egg was flattened at the region of contact with its neighbors.

I have isolated many of these eggs to see whether double embryos would result as one might have anticipated would be the case, but in a many as twenty-five isolations double embryos were never formed. Each egg segmented independently of the other — although the products of division often remained sticking closely to one another — and two blastulae were produced having separate segmentation cavities. The walls of the blastulae remained for a short time sticking to one another, but fusion of the walls never took place. It is possible that in other lots of eggs, or under other conditions, these double and treble eggs might give rise to single blastulae, but the results show that this does not usually take place.

Many of the double blastulae of the first experiment were isolated to see whether a single or a double gastrulation would take place, and to see further whether the extent to which the fusion had taken place would determine whether a single or double invagination would follow. Two invaginations always take place in the double blastulae. The positions of the regions invaginated vary and bear no fixed relation to the region of fusion of the two blastulae (Figs. 8—12). The invaginations may lie near to one another (Figs. 7, 13), or be far apart (Figs. 8, 9), their outer openings may be near together and from this point they may diverge (Fig. 13), or the outer openings may be far apart, and the inner ends of the invaginations approach one another (Figs. 8, and 10).

In one remarkable blastula, which had been isolated (Fig. 7) the two invaginations took place very near to one another and the

two tubes fused so that I thought at first that I had found a double larva with but a single invagination. Careful examination showed however that the single archenteron was in reality a double tube. On the next day the two tubes had fused into one and to all appearances a single large embryo was formed having one large digestive tract (Fig. 7). The subsequent fate of this embryo will be described below.

In several cases one archenteron invaginated before the other and produced the effect of a single invagination of the wall of the double blastula (Fig. 12). Such appearances may be misleading because when such forms are isolated it is found that another invagination appears later. Even the single (normal) eggs of this lot showed much variation in their times of gastrulating.

It is not uncommon to find that at the end of gastrulation one of the two invaginations has far outstripped the other and under these conditions the larger archenteron seems to dominate the whole mass. Around this as a center the outer wall develops while the smaller invagination may cease to grow and appears proportionately smaller and smaller in size (Figs. 11, 15, 18). A great deal of variation in the form of the skeleton is found. In those larvae where the blastulae remained separated by a constriction we find that two nearly complete skeletons develop, so that we have two united larvae with a continuous outer wall and a common segmentation cavity but with two archentera and a double skeleton. When two skeletons develop, in twins closely united, they are often so complicated that it is difficult to make out the parts belonging to each.

In those gastrulae which have been from the beginning closely united, and in which one archenteron has developed faster than the other, we find that one skeleton develops and that the other remains in a lower stage of development or develops a grotesque form (Figs. 15, 18 and 19). In the larva described above, in which the two archentera had early fused into one (Fig. 7), the skeleton developed as though the whole embryo were a single form. At two centres the radiate spicules appear and these continued to develop somewhat irregularly as shown in the figure (Fig. 16). The figure is from the preserved larva, and gives the impression of much greater irregularity of outline that really existed in the living animal. When alive and at the time when the first spicules appeared the larva appeared exactly like a normal larva and differed only in its greater size. The position of the spicules that would have extended out into the arms are not, as appears, in an abnormal position;

for occasionally in a normal larva with a normal skeleton, but in which bending of the body wall has not brought the arms and skeleton into the final position the result is the same. Comparing such a larva Fig. 17, with the figure of this double larva Fig. 16, we see, that in each the arm spicules lie in the same region of the body.

Other larvae are shown in Figs. 15 and 18. In these, one digestive tract has far out-stripped the other, and the whole structure appears to form but a single larva. These larvae are larger than the normal, but not in the same proportion as were the earlier stages. There is not the same ratio of increase in size in the double, as in the single larvae. An account of some of the details of these figures will be found in the description of the plates. In each larva the remnant of a second digestive tract is present and also a rudiment of another skeleton.

Fig. 15 is a drawing of the larva in which the two digestive tracts have equally developed, and fused for the greater part of their extent into a single tube. The skeleton present corresponds to that of a single larva. It is interesting to compare this condition with that of the other larve Fig. 14 in which a similar but less complete fusion of the digestive organs had taken place.

The phenomena in regard to the formation of the arms of the double larvae run parallel to those of the skeleton. In some cases a more or less perfect development of the arms of both larvae takes place, in others various combinations of the arms may occur. But when one archenteron, and one skeleton have developed faster than the other we find, as the figures show, only one set of arms developed. Such larvae appear like large normal forms.

Only two other instances are known to me in which an early union of embryos has been observed. METSCHNIKOFF[1]) states that the blastulae of Mitrocoma Annae may fuse together. There is no evidence furnished as to the extent of the fusion in relation to the individuality of the embryo, although a single planula results with constrictions. We do not know whether one or two hydroids would come from these double larvae.

HERBST[2]) found that the plutei of Sphaerechinus in salt solutions of given composition sometimes fused together, and concluded that

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1) Embryologische Studien an Medusen 1886, pag. 51.

2) Experimentelle Untersuchungen etc. Zeitschr. f. wiss. Zool. LV. 3, 1892.

this took place more easely between weaker larvae than between normal. It is instructive to compare LOEB's results of (fused?) plutei in this connection!

My own results differ from those of HERBST principally in that fusion takes place at a time when the cell or cells are undifferentiated. Under these conditions the material of both eggs is sometimes worked over into a single larva having more or less the perfect normal form but of large size.

BORN[1]) has recently been able to unite to one another different parts of embryos of the frog. The process of fusion here is comparable to the results of METSCHNIKOFF and HERBST, although the conditions of the experiment are different.

The observations on Sphaerechinus recorded in the preceeding pages have a direct bearing on results that LOEB has gotten on the eggs of Arbacia. LOEB placed fertilized eggs of this sea-urchin in diluted sea-water. The egg membranes burst and the protoplasm partially flowed out. LOEB states that two embryos develop under these conditions, one from the protoplasm that has remained inside the membrane, and another from the extruded protoplasm. The two embryos remain stuck together producing Siamese twins. But this is not all, for LOEB speaks of getting sometimes three such larvae from one egg and even four! Under what conditions the protruding protoplasm is divided into three or four parts is not stated. In all the cases figured by LOEB the blastulae have separate segmentation cavities. LOEB gives no measurements of these products of his experiment and no camera drawings for comparison with the normal. Consequently the reader has no means of judging of the relative sizes of the larvae.

Moreover why LOEB does not figure more than four united larvae is not clear, since he has himself shown in a later paper that much smaller portions of the protoplasm of the egg than one fourth can produce blastulae!

In one of my own experiments I got groups of larvae stuck together like LOEB's larvae. Actual measurements showed that these were produced by the fusion of individual blastulae of normal size. Chains of such compound blastulae were found united in twos, threes, fours, fives, sixes and higher numbers.

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[1]) Die künstliche Vereinigung lebender Theilstücke von Amphibien-Larven. Jahresbericht d. Schles. Gesellsch. für vaterländische Kultur. Juni 1894.

It should be noted that in one important respect LOEB's and my own experiments are alike. In both experiments the eggs are freed from their outer membranes making possible an early fusion of the naked protoplasm of eggs or blastulae lying against one another.

The indirect evidence of my experiment is strong enough, I think, to throw doubts on a part of LOEB's results. Certainly this seems true for the groups of three, and four (and more?) united blastulae. It is to be hoped that the experiment may be repeated on Arbacia, and some measurements given of the results.

Zusammenfassung.

Unter gewissen Umständen können zwei (oder mehr) Eier, wahrscheinlich auf dem Blastulastadium, mit einander verschmelzen und eine einzige mehr oder weniger kuglige Blastula bilden.

Stets finden in solcher Doppelblastula zwei Darmeinwucherungen statt. Oft überholt die eine Einwucherung die andere im Wachsthum, und um erstere als Centrum formt sich dann die ganze (doppelte) Wandung zu einer einzigen Larve um.

Ein Skelet reicht durch die ganze Länge einer solchen Larve; das Rudiment eines zweiten Skelets kann auch vorhanden sein.

Description of plate IX.

Fig. 1. Normal blastula of same age as double blastulae of Fig. 2—6. Measured $6^1/_2 \times 6^1/_2$ (3 A A). ZEISS.
Fig. 2. Double blastula. Fusion very complete.
Fig. 3. Double blastula. Fusion complete $10 \times 8^1/_2$ producing a nearly spherical form.
Fig. 4. Double blastula. Fusion not so complete as last. $10^1/_2 \times 8$ (3 A A).
Fig. 5. Double blastula. Fusion less complete; one of these may have come from an egg-fragment.
Fig. 6. Double blastula. The two constricted at region of fusion.
Fig. 7. A double gastrula. The two archentera have fused into a single tube 10×9 (3 A A). For a later stage see Fig. 16.
Fig. 8. Double gastrula with invaginated parts widely separated. $7 + \times 10$ (3 A A).
Fig. 9. Double gastrula. Invaginations just beginning. 10×9 (3 A A).

Fig. 10. Double gastrula. External openings widely separated. The inner ends of archentera fused to one another. Ectoderm shows a single thickening.

Fig. 11. Double gastrula at »prism stage«. One invagination remains small. 9×9 (3 A A).

Fig. 12. Double gastrula but only one archenteron has appeared as yet. 10×7.

Fig. 13. Double larva with two archentera 9×9 (3 A A).

Fig. 14. Double larva with the two archentera partially fused into one. A skeleton irregularly developed with beginning for four arms (not well shown in figure) $10 \times 11 \times 12$ (3 A A).

Fig. 15. Double pluteus with one well developed archenteron. Another rudimentary skeleton s^2 and a rudimentary archenteron a^2 also present. (3 C.)

Fig. 16. Double pluteus (see Fig. 7). The two archentera fused into one. Skeleton irregularly developed but showing branches for two arms. (3 C.)

Fig. 17. A single larva not normally developed for comparison with Fig. 16. (3 C.)

Fig. 18 A B. Double pluteus, seen before and behind. One normal skeleton and archenteron and another rudimentary skeleton and archenteron. (3 A A.)

Fig. 19. Double pluteus. The two archentera irregularly fused, but not touching mouth thickening. A normal skeleton present also a rudiment of another. (3 A A.)

Fig. 20. Two eggs (unsegmented) stuck together after shaking (not drawn to scale).

Fig. 21. Three eggs (unsegmented) stuck together (not drawn to scale).