MOSAIC DEVELOPMENT IN ASCIDIAN EGGS

BY

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WITH 82 FIGURES.

In almost every instance in which fragments of eggs or isolated blastomeres have been found to be capable of giving rise to entire larvæ the substance of the unsegmented egg is apparently undifferentiated and the cleavage cells are so nearly equal and homogeneous that it has not been possible to trace the lineage of individ-

ual blastomeres throughout the development. The most notable exception to this rule is found in the case of ascidians. That the cleavage of the egg in these animals is constant in form and differential in character and that specific blastomeres are destined in the course of normal development to give rise to specific parts of the larva has been demonstrated by Van Beneden and Julin, Chabry, Castle, and many others. Chabry ('87) also showed, in one of the earliest experimental investigations dealing with the potency of cleavage cells, that individual blastomeres of Ascidia aspersa always develop into those parts of the larva which they would produce under normal conditions. On the other hand, Driesch ('95) discovered, some eight years later, that in Phallusia mammilata individual blastomeres up to the 4-cell stage at least are capable of giving rise to entire larvæ and this conclusion was afterward confirmed by Crampton ('97) in the case of Molgula manhattensis. Since the results of Chabry were thus flatly contradicted by these later investigators and as they have been defended by no one who has actually experimented on these eggs[1] these results have been generally discredited and the ascidians are now commonly regarded as belonging to that group of animals in which the early cleavage cells are equipotential. The ascidians, therefore, should afford an excellent opportunity of determining the exact method by which an egg fragment or isolated blastomere gives rise to an entire larva, since in this case it is possible to follow the lineage of individual cells until they enter into larval organs; furthermore, they should afford means of testing the justice of the distinction which has been proposed (Conklin, '97) between determinate and indeterminate types of cleavage, and finally they should throw light upon the significance of the high degree of differentiation which is known to exist in the early development of these animals.

I. NORMAL DEVELOPMENT.

I have recently ('05[1]) shown that these differentiations of the ascidian egg are much greater than has heretofore been supposed; in the unsegmented egg of Cynthia (Styela) partita at least five distinct kinds of oöplasm can be recognized. These are, (1) the

[1]Several persons, viz: O. Hertwig ('92), Roux ('92), Weismann ('92), Barfurth ('93) have discussed Chabry's work from a critical point of view.

deep yellow protoplasm which later enters into the muscle cells of the tail of the larva; (2) the light yellow material which becomes mesenchyme; (3) the light gray material which forms the chorda and neural plate; (4) the slate gray substance which becomes endoderm, and (5) the clear transparent protoplasm which gives rise to the general ectoderm. All of these substances are recognizable in the egg before the first cleavage and immediately after that cleavage they all occupy their definitive positions in the egg, the yellow protoplasm forming a yellow crescent around the posterior side of the egg just dorsal to the equator, the light gray substance forming a gray crescent around the anterior border of the egg, the slate gray substance lying at the middle of the dorsal hemisphere and between the two crescents, while the transparent protoplasm is chiefly localized in the ventral hemisphere of the egg. In these positions and from these substances the organs and germinal layers specified arise.

At the first cleavage of the egg all of these substances and areas are equally divided, since this cleavage lies in the plane of bilateral symmetry of the egg and future embryo. The second cleavage plane is perpendicular to the first and separates the gray crescent in front from the yellow crescent behind; the cells of the anterior quadrants are therefore very unlike the posterior ones and the two can always be distinguished at a glance. (Fig. 1.) The third cleavage is equatorial and separates four clear ventral cells from four dorsal ones which contain the yellow and gray crescents and the deep gray material. (Fig. 2.) The ectoplasm is now completely segregated in the four ventral cells but the other oöplasmic substances are not as yet located in separate cells, though from the time of the first cleavage onward their locations and boundaries are perfectly sharp and distinct.

At the fourth cleavage each of the eight cells divides, thus giving rise to sixteen cells (Fig. 3) and at the fifth cleavage these are increased to thirty-two. During the fifth cleavage the substance of the gray crescent is segregated into four cells ($A^{6.2}$, $A^{6.4}$, Fig. 4)[1] at the anterior border of the egg, while the yellow crescent comes

[1]The system of cell nomenclature employed in this paper is similar to that used by Castle ('96) and is fully explained in my work on the cell-lineage ('05[1]); in brief *A* and *a* designate cells of the anterior half of the egg, *B* and *b* those of the posterior half, the capitals being used for cells of the vegetal (dorsal) hemisphere, the lower case for those of the animal (ventral) hemisphere. Corresponding cells of the right and left sides receive the same designation, except that those of the right side are underscored.

NORMAL DEVELOPMENT OF CYNTHIA PARTITA, 4-CELL TO 64-CELL STAGES; × 333.

The yellow crescent which surrounds the posterior half of the egg dorsal to the equator is stippled. The gray crescent around the anterior border of the egg is left unshaded. The boundary between the clear protoplasm and the yolk is indicated by a crenated line. The polar bodies (shaded by vertical lines) lie at the animal or ectodermal pole.

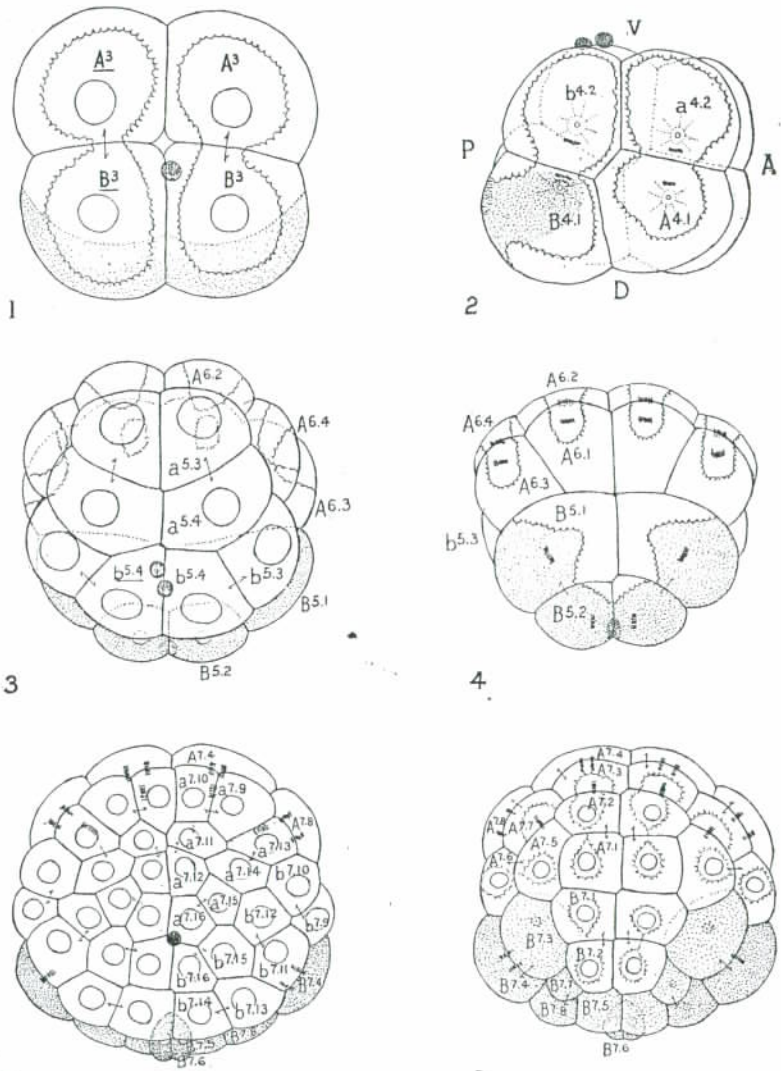
Fig. 1. Four-cell stage from the animal pole, the yellow crescent showing through the egg.

Fig. 2. Telophase of the third cleavage (8-cell stage), from the left side.

Fig. 3. Twenty-cell stage from the animal (ventral) pole.

Fig. 4. Twenty cells, transitional to the 24-cell stage, from the vegetal (dorsal) pole. The gray crescent is now segregated in the two pairs of cells $A^{6.2}$, $A^{6.4}$; the yellow crescent will be localized in separate cells at the close of the division which has already begun in the cells $B^{5.1}$.

Figs. 5 and 6. Ventral and dorsal views of the same egg in the 64-cell stage. The yellow and the gray crescents each consist of a double arc of cells; the anterior arc of the gray crescent ($A^{7.4}$, $A^{7.5}$) is composed of neural plate cells, the posterior arc ($A^{7.8}$, $A^{7.7}$), of chorda cells; only two pairs of cells in the yellow crescent ($B^{7.4}$, $B^{7.8}$) are muscle cells, the others are mesenchyme. The pair of cells $A^{7.6}$ also gives rise to mesenchyme. All the other cells of the dorsal hemisphere (Fig. 6) are endodermal. All the cells shown in Fig 5, except those of the yellow and gray crescents, are ectodermal.



to occupy six cells ($B^{6.3}$, $B^{6.4}$, $B^{6.2}$) around the posterior border (the spindles which lead to the formation of these six cells are indicated in Fig. 4). These thirty-two cells are increased to sixty-four at the next cleavage (Figs. 5 and 6); during this cleavage four chorda cells ($A^{7.3}$, $A^{7.7}$) are separated from the four neural plate cells ($A^{7.4}$, $A^{7.8}$, Fig. 6), while the six cells of the yellow crescent have given rise to twelve, four of which are muscle cells ($B^{7.4}$, $B^{7.8}$) and eight mesenchyme ($B^{7.3}$, $B^{7.7}$, $B^{7.5}$, $B^{7.6}$). At the same time an additional pair of mesenchyme cells ($A^{7.6}$) is separated from a pair of endoderm cells in the anterior quadrants. This is the only mesenchyme cell derived from the anterior quadrants.

At this stage all the substances of the germ layers and of the principal organs of the larva are gathered into separate cells, but although this segregation into separate cells comes relatively late in the cleavage these substances have been definitely localized in certain regions of the egg from the time of the first cleavage. Subsequent cleavages lead to changes in the shape of the embryo but produce no changes in this localization.

In the gastrulation the endoderm cells are depressed and are overgrown in front by the chorda cells and these in turn are covered by the neural plate cells; similarly the mesenchyme cells overgrow the endoderm at the posterior border of the blastopore, while the mesenchyme cells are overgrown by the muscle cells, and finally the latter by the ectoderm. (Figs. 7–10.) In the closure of the blastopore the anterior (dorsal) lip grows posteriorly until it covers most of the dorsal face, while the muscle cells form the lateral boundaries of the blastopore. (Figs. 9, 10.) In this overgrowth of the dorsal lip the chorda cells which originally lay at the anterior border of the egg are carried back into the posterior half of the embryo, where by interdigitation they form the chorda. The neural plate cells are also carried back with the chorda nearly to the posterior end of the embryo. The ventral (posterior) lip of the blastopore then grows forward over the remnant of the blastopore and the neural plate is rolled up into a tube which closes from behind forward. The muscle cells become arranged in three rows on each side of the chorda; in front of the muscle cells is a mass of small mesenchyme cells, while a double row of endoderm cells ventral to the chorda constitutes the cord of ventral or caudal endoderm. (Figs. 11 and 12.) Finally the tail of the larva elongates greatly and becomes coiled around the body of the larva within the egg membranes, and about twelve hours after the fertilization of the egg the larva may hatch and become free swimming. However, in a considerable proportion of cases the larva never hatches but undergoes its metamorphosis within the egg membranes.

II. OBJECTS AND METHODS OF EXPERIMENT.

This brief review of the normal development[1] shows that there is a remarkable degree of differentiation and localization of the substances of the egg and embryo and it seems to render necessary some further explanation of the results of the experiments of Driesch and Crampton; certain it is that the egg is highly differentiated and if portions of this differentiated oöplasm may give rise to portions of the larva which they would never produce under normal conditions it is important to know the steps by which this is accomplished.

With this object in view I spent the summer of 1904 at the Marine Biological Laboratory at Woods Hole, Mass., experimenting on the eggs of Cynthia (Styela) partita and of Molgula manhattensis; I was unable to obtain Ciona intestinalis, the normal development of which I had studied during the previous summer, and my experimental work is therefore limited to the two species first named. Most of my work was done on the egg of Cynthia, which is a better object for experimental work than that of Molgula, owing to its greater size and the more brilliant coloring of its different oöplasmic substances. Enough work was done on Molgula, however, to show that the development of isolated blastomeres is the same in this genus as in Cynthia.

All the experiments performed had for their purpose the testing of the potencies of the various substances and blastomeres of the egg. Injuries to the unsegmented egg of whatever nature, whether produced by sticking, cutting or shaking the eggs, invariably inhibited all further development. I have therefore been unable to test the developmental potencies of the different kinds of oöplasm of the unsegmented egg. But inasmuch as these substances are the same in appearance and localization before and

[1]For a more detailed account of the normal development of these ascidians the reader is referred to my previous papers on the "Organization and Cell-Lineage of the Ascidian Egg" ('05[1]), and on "Organ-Forming Substances in the Eggs of Ascidians" ('05[2]).

NORMAL DEVELOPMENT OF CYNTHIA PARTITA, GASTRULA TO TADPOLE; × 333.

The neural plate or tube is finely stippled, the chorda coarsely stippled; muscle cells are shaded by vertical lines, mesenchyme by transverse lines.

Figs. 7 and 8. Ventral and dorsal views of a gastrula (180-cell stage), showing T-shaped blastopore, neural and chorda plates, mesenchyme and muscle cells. Most of the cleavage cells are in the ninth generation.

Figs. 9 and 10. Two views of the same gastrula from the dorsal pole; Fig. 9, showing the superficial cells, Fig. 10, those at a deeper level. The overgrowth of the dorsal lip of the blastopore and the approximation of the muscle cells of each side toward the median plane have reduced the blastopore to a longitudinal groove in the posterior half of the embryo. The ectoderm cells are in the tenth generation and there are in the entire embryo about 360 cells.

Fig. 11. Dorsal view of an embryo in which the neural plate (n. p.) is closing to form the neural tube (n. t.) Beneath the nerve tube is the notochord and on each side of the latter is shown a row of muscle cells (ms.) At the posterior end of the muscle rows is the caudal mesenchyme, at their anterior end the trunk mesenchyme (m'ch.)

Fig. 12. Young tadpole viewed from the left side, showing three rows of large muscle cells (ms.) along the side of the notochord (ch.); dorsal to the latter is the nerve tube (n. t.); anterior to the muscle rows is the trunk mesenchyme (m'ch.); ventral to them is the ventral or caudal endoderm (v. end.)

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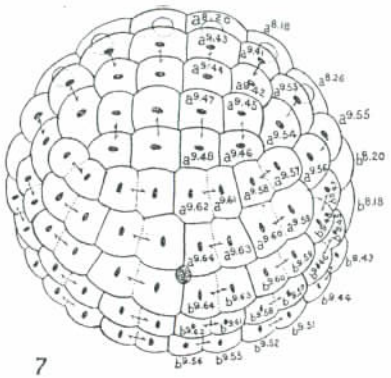

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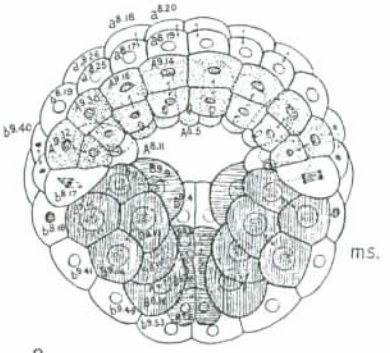

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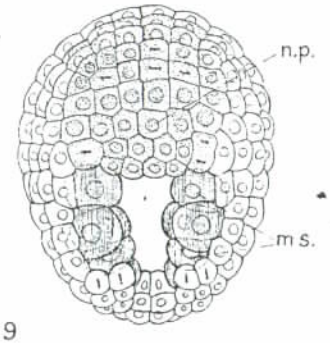

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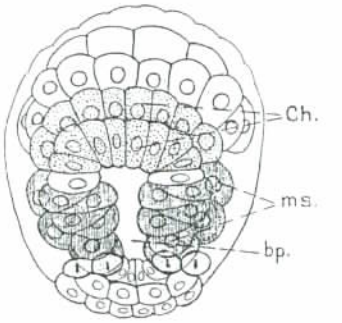

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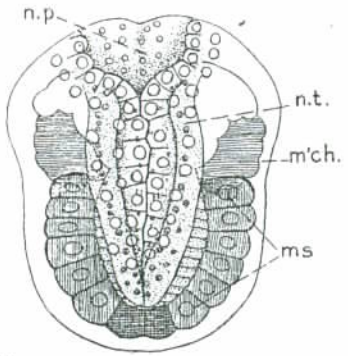

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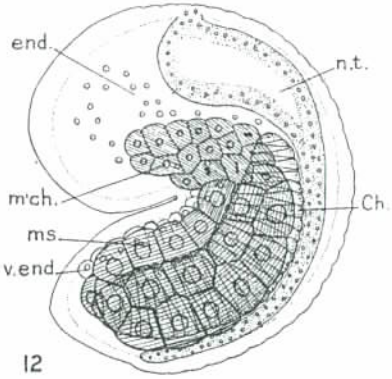

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after cleavage begins it can scarcely be doubted that their potencies are also the same. Hundreds of experiments involving many thousands of eggs were made upon the various cleavage stages. The methods of experimenting which I employed were essentially like those used by Driesch and Crampton, viz: the eggs in the 2-cell, 4-cell, 8-cell or later stages were strongly spurted with a pipette, or were shaken in a vial, and thereby some of the blastomeres were frequently injured while others were uninjured and continued to develop. The injured blastomeres were rarely killed, as was shown by the fact that they remained transparent and entire for a day or more, whereas dead cells soon become opaque and disintegrate. These injured cells never again divide and sections show that their nuclei are frequently broken and their chromosomes scattered. Cells are more likely to be injured during nuclear division than during rest. The fact that these injured cells never again divide though they remain whole within the chorion and preserve their characteristic color and structure makes it possible to determine at all stages just what cell or cells have been injured. Whether or not the presence of these injured cells within the chorion may influence the development of the uninjured cells will be considered later. Attempts to completely separate individual blastomeres by the use of Herbst's calcium-free sea water were not successful, probably owing to the presence of the chorion and to the close union between the blastomeres.

In addition to this method of experimentation which yielded hundreds and thousands of eggs in which one or more of the blastomeres had been injured I also cut eggs and embryos in two with knives made from small needles. In no single instance was I able to get fragments of unsegmented eggs to develop; in the gastrula stages I was more successful, being able to cut gastrulæ in two in the manner described by Driesch ('03) and observe the subsequent development.

I have not attempted to repeat the various ingenious methods of injuring blastomeres which were devised and employed by Chabry, since they are necessarily slow and difficult of application and yield but a small number of injured eggs, whereas by simply spurting or shaking the eggs one may injure blastomeres in an enormous number of eggs which can then be sorted out and classified according to the character of the injury; furthermore the ease and certainty with which the identity of injured blastomeres of

Cynthia may always be determined renders unnecessary such experiments as Chabry's on the individual cleavage cells.

If one desires to trace with accuracy the lineage of individual blastomeres, whether in normal or experimentally altered development, it is essential that a large quantity of material should be available. In even the most favorable material the lineage of the later stages can be successfully studied only by the aid of fixed and stained material and without a large number of eggs it is difficult if not impossible to secure all the stages of development. Furthermore it is desirable that a considerable number of eggs of every stage be available for study, since the liability to error decreases with the number of cases studied. Accordingly, in addition to the study of living eggs during successive stages after their injury, many eggs were also fixed at brief intervals and were afterward stained and mounted entire or sectioned. For this purpose I have found Kleinenberg's picro-sulphuric acid followed by my picro-hæmatoxylin to give the best results. Entire eggs so prepared show cell outlines, nuclei and karyokinetic figures much more plainly than in the living condition; on the other hand the yellow crescent is less distinct since the yellow pigment is extracted by alcohol; nevertheless this crescent may always be recognized by its peculiar staining qualities and it therefore affords a never failing aid in orientation.

III. RESULTS OF EXPERIMENTS.

In undertaking this work it seemed to me scarcely possible that all of these strikingly different kinds of oöplasm, each with its own peculiar developmental history and destiny, were nevertheless morphogenetically alike, as might be concluded from the results of Driesch and Crampton. On the other hand a possible escape from this conclusion was suggested by the fact that although the cleavage cells are strikingly different from one another, the isolation of the oöplasmic substances in them is not quite complete; almost all of the yellow protoplasm is contained in the yellow crescent; but a small amount of it is found around the nuclei of all the cells; most of the gray substance is contained within the dorsal hemisphere, but a small amount of it occurs in the ventral cells also; most of the clear protoplasm is found in the ventral hemisphere but a small quantity is also found in the dorsal cells.

It therefore seemed possible that the production of a complete larva from any one or two of the first four cells might be due to the replacing of a missing substance by the greater development of the trace of that substance contained in the cells in question. Thus the anterior quadrants which lack the yellow crescent might, perhaps, regenerate it from the small amount of yellow perinuclear protoplasm which they contain, and correspondingly the posterior quadrants might regenerate the lacking gray crescent from the small amount of gray substance which they contain. In the light of the work of Driesch and Crampton either there must be such regeneration, or the substances which appear so different must after all be each and all totipotent.

However the solution of this problem has turned out to be much simpler than I had supposed possible, viz: *isolated blastomeres do not give rise to entire larvæ, as claimed by Driesch and Crampton, but on the contrary each blastomere produces only those parts of a larva which would arise from it under normal conditions. The development is, in short, a "mosaic work."* Since the first cleavage is bilaterally symmetrical each of the first two blastomeres contains one-half of each and all of the substances of the egg and correspondingly the half larva which develops from one of these blastomeres contains portions of every larval organ. Owing to the fact that the cells which arise from an isolated blastomere close over the injured surface these partial embryos are rounded in form and many of the one-half larvæ resemble superficially whole larvæ of half size, but in no case are they complete. When the anterior or posterior quadrants of the 4-cell stage are killed nothing even remotely resembling a normal larva is ever produced. My results are therefore directly opposed to those of Driesch and they agree in all essential respects with those of Chabry.

The partial embryos and larvæ obtained in these experiments may be classified as right or left, anterior or posterior, dorsal or ventral, or composite forms. Furthermore they may be known as half, quarter, eighth, sixteenth, etc., embryos, according as they are produced from blastomeres of the 2, 4, 8, 16, etc., cell stages; however, the character of the embryo depends entirely upon the region from which the isolated blastomeres come and not upon the number of such blastomeres.

1. *Right or Left Half Embryos (Figs. 13–33, 36–46).*

a. Cleavage.

When the right or left half of an egg is injured in the 2, 4 or 8-cell stage, the other half continues to segment in a normal manner, provided it was not also injured. I have traced the cell-lineage of these right or left half embryos up to the eighth generation of cleavage cells (the 112-cell stage of normal eggs), while I have determined the lineage of many individual cells as late as the ninth or tenth generation (218–360 cell-stage). The cell-lineage of these half embryos is essentially like the right or left half of a normal egg, except that the direction of division and consequently the position and size of some of the blastomeres may be slightly altered.

This alteration in the direction of cleavage is most evident in cases where the egg was injured in the 2-cell stage, and it is probably due to the fact that the uninjured blastomere in such cases becomes nearly spherical in shape, and does not remain hemispherical as in the normal egg. Owing to this fact the median pole of certain cleavage spindles, *i. e.*, the one next to the original median plane, is shifted toward the middle of that plane. The resulting mass of cells is, therefore, more nearly spherical than in the half of a normal embryo. (Figs. 13–20.) If the injury occurs in the 4-cell stage or later, the change in the direction of the early cleavages is not so evident as when it takes place in the 2-cell stage. In case one of the blastomeres was injured at the close of the first cleavage, the direction of the karyokinetic spindles of the second and third cleavages are entirely normal, since in both these cases they lie parallel with the first cleavage plane, Fig. 13; but in the fourth cleavage in which one pole of the spindles lies nearer that plane than the other, the median pole is shifted toward the middle of that plane and consequently the cells formed along the median plane come into closer contact with one another and the cell aggregate is more nearly spherical than in the right or left half of a normal 16-cell stage. (Figs. 14, 15, 21, 22.) These results entirely agree with those of Chabry and Crampton.

The fifth cleavage of the right or left half embryo is also like the normal except in the direction of a few of the divisions; *e. g.*, Fig. 16 is nearly normal but in Fig. 17 the division of the cell

DEVELOPMENT OF RIGHT BLASTOMERE OF 2-CELL STAGE.

Figs. 13–20. Successive stages in the development of the same right half embryo, the left blastomere having been injured in the 2-cell stage; drawn at intervals of about five minutes. Here and elsewhere the yellow protoplasm is indicated by coarse stipples.

Fig. 13. Right half of 8-cell stage, posterior view. A small amount of yellow protoplasm surrounds the nucleus of the ectoderm cell $b^{4.2}$. The position of the cells shows that the ventral ends of the third cleavage spindles diverged from the first cleavage plane in the posterior quadrant and converged toward that plane in the anterior quadrant, just as in the normal egg. (See Conklin, '05[1].)

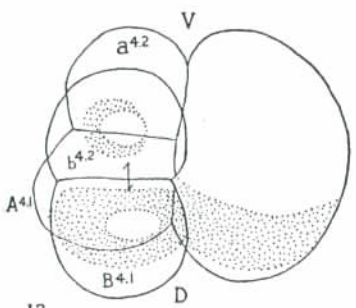
Fig. 14. Right half of 16-cell stage, anterior view. The yellow crescent is seen through the cell $B^{5.1}$. In the normal egg of this stage the cells $A^{5.1}$ and $a^{5.3}$ lie more nearly in front of the cells $A^{5.2}$ and $a^{5.4}$.

Fig. 15. Same stage as preceding posterior view. In normal eggs the cells $B^{5.2}$ and $b^{5.4}$ lie nearly behind the cells $B^{5.1}$ and $b^{5.3}$ and not on their median side.

Fig. 16. Right half of 30-cell stage, dorsal view. $A^{6.2}$ and $A^{6.4}$ are cells of the gray crescent; $B^{6.2}$ and $B^{6.3}$, cells of the yellow crescent.

Fig. 17. Right half of 34-cell stage, posterior view. In normal eggs the cell $B^{6.4}$ lies on the lateral border of $B^{6.3}$.

Fig. 18. Same stage as preceding, dorsal view. The cell $B^{6.1}$ normally lies between $B^{6.3}$ and $A^{6.1}$.



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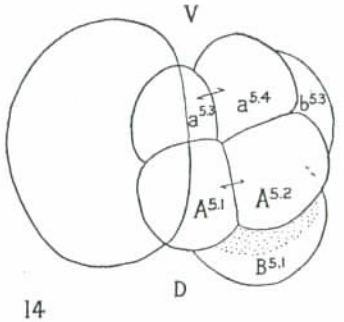

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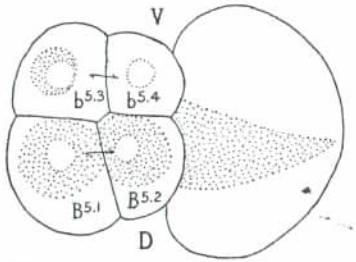

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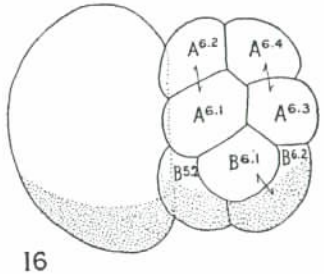

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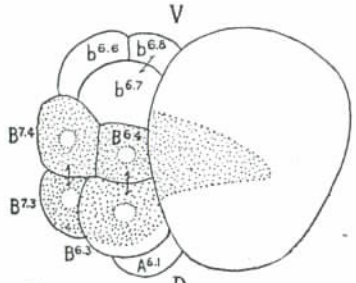

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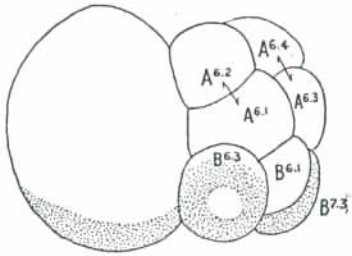

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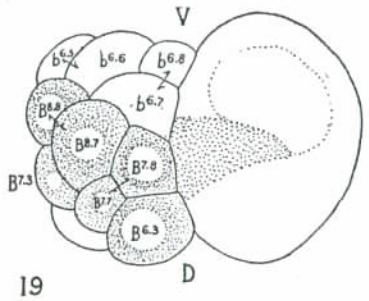
DEVELOPMENT OF RIGHT BLASTOMERE OF THE 2-CELL STAGE; ALSO OF RIGHT AND LEFT BLASTOMERES OF THE 4-CELL STAGE.

Figs. 19, 20. Same embryo as that shown in Figs. 13–18. Fig. 19. Right half of 46-cell stage, posterior view; the yellow crescent cells are not quite normal in position. Fig. 20. Right half of 48-cell stage, dorsal view. The caudal endoderm cells ($B^{7.1}$ and $B^{7.2}$) have been shoved away from the median plane by the cell $B^{7.8}$.

Figs. 21, 22. Fixed and stained preparations of half embryos in the 16-cell stage. Fig. 21. Right half embryo, posterior view. Fig. 22. Left half embryo, ventral-posterior view.

Figs. 23, 24. Successive stages of one and the same half embryo, the left half having been injured in the 4-cell stage, dorsal view. Fig. 23. Right half of 16-cell stage. Fig. 24. Right half of 32-cell stage. The cleavage is like the right half of a normal egg in every respect.



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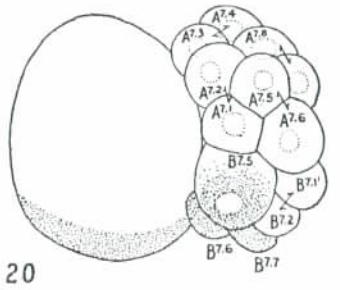

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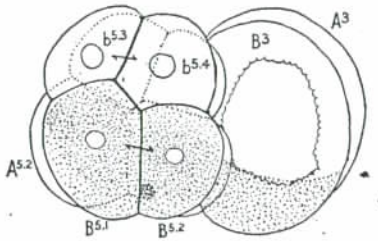

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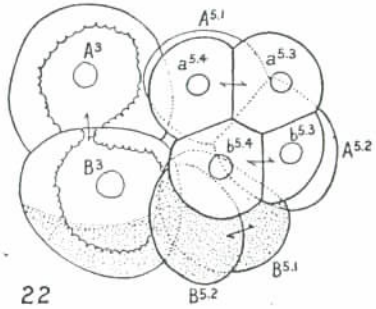

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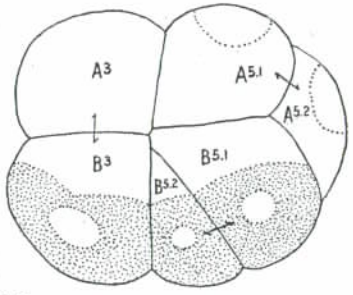

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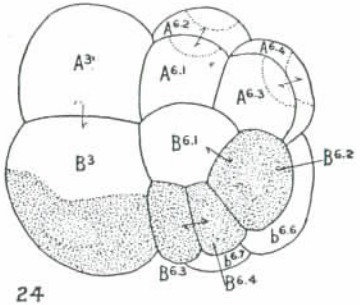

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$B^{5.2}$ into $B^{6.3}$ and $B^{6.4}$ is almost at right angles to its normal direction. In other cases, as is shown in Fig. 24, this cleavage is normal in direction, and I am, therefore, of the opinion that the condition shown in Fig. 17 and the later stage of this same egg shown in Fig. 19 may be due to some slight injury to the developing half of this egg. In Fig. 18, which is a dorsal view of the same egg in the same stage as Fig. 17, the cells $A^{6.2}$ and $A^{6.4}$ have moved in toward the median plane as compared with Fig. 16, though in this respect, also, the corresponding stage shown in Fig. 24 is quite normal. This shifting of the anterior dorsal cells toward the median plane is shown again at the next cleavage (the sixth), of this egg. (Fig. 20.)

The seventh cleavage, which is shown in Figs. 25 and 26, is also normal except for the direction of a few of the divisions. The cells which constitute the yellow and gray crescents are in all respects like the right half of a normal egg. However the position of the cells $A^{7.1}$ and $A^{7.2}$, Fig. 25, and the direction of division in several of the ectoderm cells shown in Fig. 26 are not quite normal.

In conclusion therefore it may be said that the cleavage of one of the blastomeres of the 2-cell stage or of the right or left blastomeres of the 4-cell stage, is like that of the corresponding half of a normal egg, except in minor details. Even these minor differences are not always present and when they are they do not alter the localization of the oöplasmic substances. In every case the distribution of the yellow, the gray and the clear substances to the different blastomeres is the same as in the right or left half of a normal egg; the cells of the yellow crescent, for example, form only the right or left half of a normal crescent, and the same is true of the gray crescent and of the other substances of the egg. Even the small amount of yellow protoplasm which is found around the nuclei of the posterior ectoderm cells $b^{4.2}$, Fig. 13, is perfectly normal in its occurrence and subsequent distribution.

I have elsewhere ('05[1]) shown that the localization of different oöplasmic substances in the ascidian egg precedes cleavage and that cleavage and localization are here relatively independent of each other; these experiments show that in both cleavage and localization the development of the right or left half of an ascidian egg is a "mosaic work," for the slight amount of regulation, which is manifested in the changes in the direction of certain cleavages, and the consequent closing of the embryo in no way alters the

histological character of the cleavage cells nor their developmental tendencies.

b. Gastrulation.

In the development of the right or left half of an egg the process of gastrulation sometimes occurs in an unusual manner. The most frequent modification of the normal process is that shown in Figs. 27, 29, 30, where the endoderm cells are not infolded but come to protrude above the level of the other cells, thus forming exogastrulæ. In later stages these endoderm cells must become infolded for it is a rare thing to see exogastrulæ or any indication of an original evagination of endoderm cells in any of the cultures of older embryos. By what process these exogastrulæ right themselves I have not been able to observe, but I think it probable that this like normal gastrulation is accomplished by overgrowth of the ectoderm cells and change of shape of the endoderm cells.

Sometimes when the endoderm cells are evaginated other portions of the blastula wall invaginate. In this way false gastrulæ may arise in which the infolded cells are not endodermal but ectodermal, as is clearly shown by their histological structure. (Fig. 63.)

While some embryos in the gastrula stage show such abnormalities as those which have just been described in other cases the gastrula is strictly a half one, as is shown in Fig. 31, and it seems to me probable that exogastrulæ or false gastrulæ only arise when the surviving half of the egg has been slightly injured. These half gastrulæ contain just one-half of all the cells of the normal gastrula and the position of the various cells and organ bases is essentially like that which occurs in the right or left half of a normal gastrula; the cells of the yellow crescent lie along one side only of the blastopore groove; the neural plate and chorda cells each form half of the arc which is normally present in the anterior lip of the blastopore, while the closing of the open side of the gastrula, which is turned toward the injured cell, is chiefly accomplished by the overgrowth of the ectoderm cells of the ventral side. (Fig. 31.)

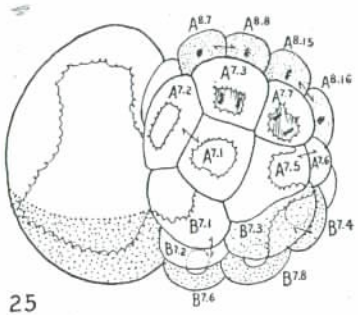
Except, therefore, for this tendency of the cells along the injured side to come together, these half gastrulæ are strictly partial and the gastrulation no less than the cleavage may be regarded as an illustration of mosaic development.

RIGHT OR LEFT HALF EMBRYOS; 64-CELLS TO GASTRULA.

Figs. 25, 26. Fixed and stained half embryos; spurted in the 2-cell stage and fixed 2 hours later. Fig. 25. Right half of 64–76-cell stage, dorsal view. The neural plate cells ($A^{8.7}$, $A^{8.8}$, $A^{8.15}$, $A^{8.16}$) have just divided, the chorda cells ($A^{7.3}$, $A^{7.7}$) are dividing. The position of the cells $A^{7.1}$, $A^{7.2}$ is slightly abnormal. (*v.* Fig. 6.) Fig. 26. Left half of 64–76-cell stage, ventral-posterior view.

Figs. 27, 28. Right half of embryo in about 180-cell stage; spurted in the 4-cell stage and fixed 2½ hours later. Fig. 27. Dorsal view; the large endoderm cells lie above the level of the other cells and form an exogastrula; some of the yellow cells (stippled) still lie at the surface while others are covered by endoderm cells. Fig. 28. Ventral view of similar embryo.

Figs. 29, 30. Living right half embryos, dorsal view, showing the endoderm cells forming exogastrulæ and the yellow crescent cells at the surface.



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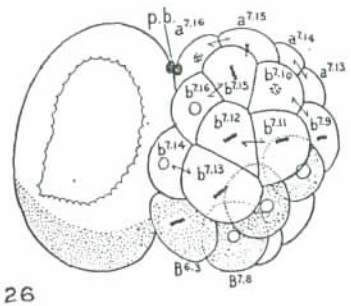

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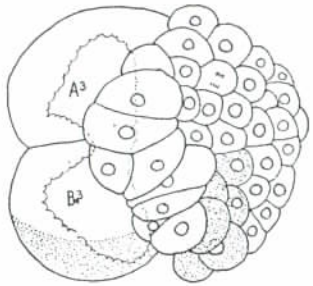

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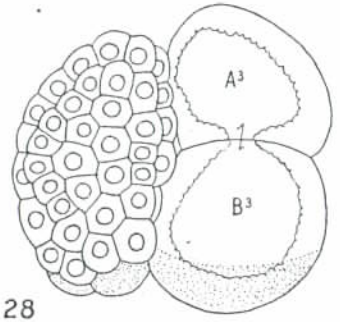

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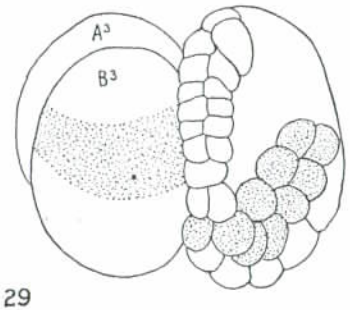

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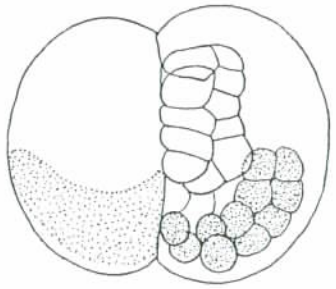

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RIGHT OR LEFT HALF OR THREE-QUARTER EMBRYOS; GASTRULA TO TADPOLE. DRAWN FROM FIXED AND STAINED MATERIAL.

Fig. 31. Right half gastrula of about 220-cell stage; spurted in the 4-cell stage and fixed 3 hours later. The neural plate, chorda and mesoderm cells are present only on the right side and in their normal positions and numbers.

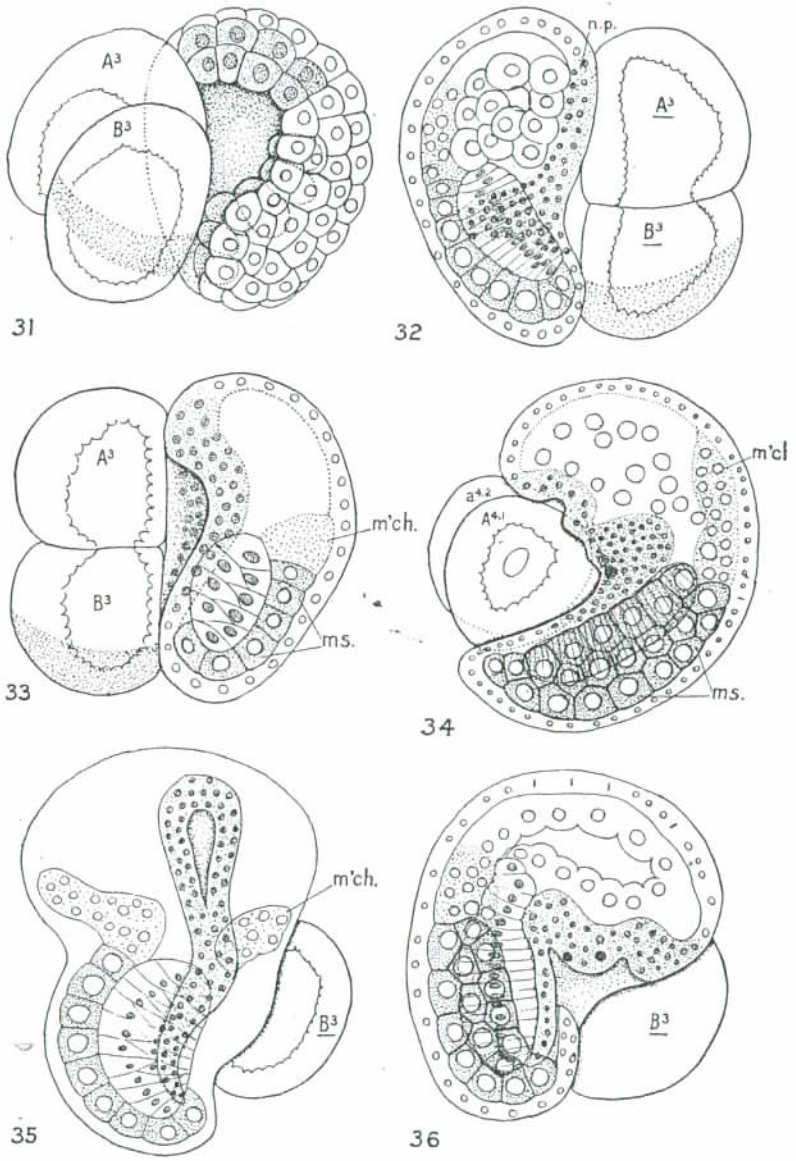
Fig. 32. Left half of young tadpole, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. The notochord is normal except for size and number of cells; the muscle and mesenchyme cells are present only on one side; the neural plate is abnormal in form but not in position.

Fig. 33. Right half of young tadpole, dorsal view; spurted in the 4-cell stage, fixed 4½ hours later (slightly younger stage than Fig. 32). The notochord consists of a small number of cells which are interdigitating; muscle cells and mesenchyme lie on the right side of the notochord, but not on the left, though the muscle cells have begun to grow around to the left side; the neural plate is normal in position but not in form.

Fig. 34. Right-posterior three-quarter embryo, from the right side. The left anterior cells ($A^{4.1}$, $a^{4.2}$) were killed in the 8-cell stage and the embryo fixed 5 hours later. The posterior half of the embryo is normal, but the left half of the anterior part is lacking and the neural plate is abnormal and has not formed a tube though sense spots are present.

Fig. 35. Left-anterior three-quarter embryo, dorsal view; the right posterior quadrant (B^3) was killed in the 4-cell stage and the embryo fixed 6 hours later. The anterior half of the embryo is entirely normal. The muscle cells are lacking on the right side though they have begun to grow around the hinder end of the notochord. The posterior portion of the trunk mesenchyme is found only on the left side, but its anterior portion, which is derived from the cells $A^{7.6}$ and $A^{7.8}$ (Fig. 6) of the anterior quadrants is present on both sides. In the region of the injured cell the notochord and neural tube are curved away from that cell.

Fig. 36. Left half embryo, from left side; spurted in the 4-cell stage, fixed 6 hours later. The dorsal lip of the blastopore is being overgrown by the ventral (posterior) lip. Muscle cells and mesenchyme are found only on the left side. The neural plate is abnormally folded, but still open; sense spots are present.



c. Formation of Larva.

A considerably later stage in the development of the half embryo is shown in Figs. 32, 33 and 36 (Figs. 34 and 35 are three-quarter embryos and will be described later); of these stages Fig. 33 is the youngest and Fig. 36 the oldest. In all of these figures the blastopore has already closed and the chorda cells have given rise to a fusiform notochord, which lies in the posterior half of the embryo. The blastopore closes chiefly by the posterior growth of the dorsal (anterior) lip, as in the normal gastrula. With the formation of the notochord the posterior half of the embryo becomes elongated and narrower than the anterior half and the developing tail bends around toward the injured side. (Figs. 32, 33.)

The anterior half remains large, the posterior half becomes long and narrow; the latter portion contains the notochord and muscle cells, the former the gastral endoderm, mesenchyme and most of the neural plate. The general superficial appearance of an embryo of this stage is very similar to a normal one, but a more detailed study shows many differences.

(*1*) *Neural Plate.* The neural plate occupies in the main its normal position, that is, it lies along the first cleavage plane on the dorsal side, next to the injured cell. In this position the plate becomes folded and ultimately comes to contain a vesicle (the sense vesicle) though the steps by which this vesicle is formed are always irregular and abnormal. (Figs. 36–40.) The anterior portion of the plate is usually doubled over posteriorly while the posterior portion is folded forward (Figs. 36, 39, 40) and in this way a vesicle is finally formed.

The tail of the embryo grows around toward the injured side so that the concave side of the embryo is median or dorsal, the convex side being lateral or ventral. In the younger, normal larvæ the concave side is ventral, the convex dorsal. In these half larvæ the nerve plate lies along the concave side, a condition which is the reverse of what is found in the normal larva. (*cf.* Figs. 12 and 36.) In the older half larvæ there is almost always found one or more pigmented sense spots in the neural plate or sense vesicle. (Figs. 36–40, 45, 46.) These pigment spots appear within cells of the neural plate and, as I am well convinced, always within definite cells, though owing to the abnormal foldings of the neural plate they do not always occupy exactly the same

positions. Furthermore these sense spots may be more numerous than in the normal larva, as shown in Figs. 45 and 46, probably owing to the fact that the cells which form the pigment and which normally lie on the margins of the neural plate do not come together to form two spots as in normal larvæ, but remain separated so that several such spots are formed.

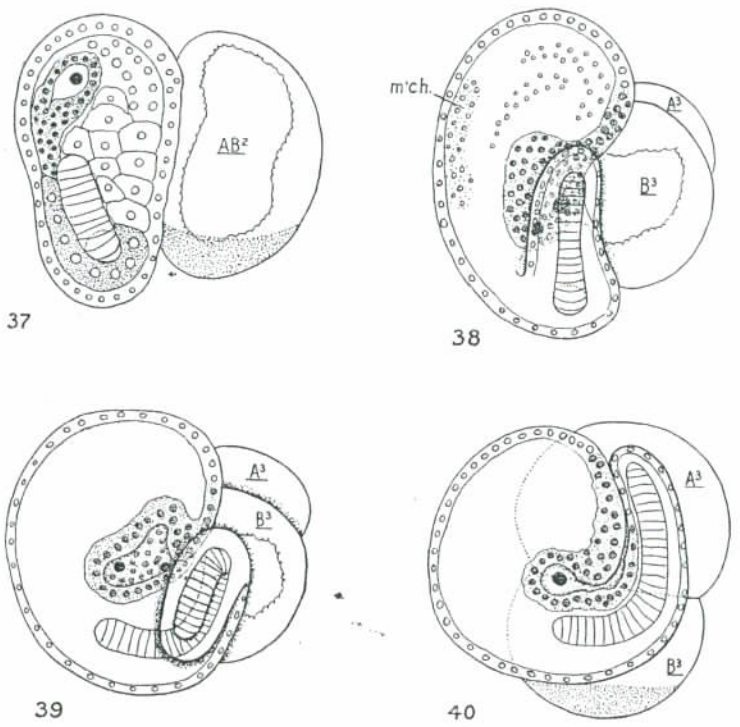
(*2*) *Notochord.* The chorda cells grow back into the posterior half of the embryo and the cells here interdigitate in the normal manner, finally forming a linear series of cells. (Figs. 32–46.) The notochord, which is at first relatively short and thick, Fig. 33, becomes later very much longer and more slender, Fig. 40, and in all respects it has the appearance of a normal notochord, save that it evidently contains a smaller number of cells. The position of the notochord of the half larva is always slightly abnormal; it never lies along the original median plane (first cleavage) as in normal larvæ, but its anterior end is diverted away from that plane and toward the lateral border of the larva. (Figs. 32, 33, 37, 41.) This position is that which the chorda cells, which arise in the anterior lip of the blastopore and which grow posteriorly around the margin of the blastopore, would naturally assume. (*cf.* Figs. 31 and 33.) What it is which causes the chorda cells to interdigitate in their characteristic manner is a question difficult to answer; it certainly is not dependent upon the crowding together of chorda cells from the right and left sides since it occurs normally when the cells of one side only are present; on the other hand it must depend upon a certain amount of lateral compression of the chorda cells since it occurs very rarely if at all in the anterior half larvæ in which the ectoderm and mesoderm of the tail are lacking.

(*3*) *Muscles and Mesenchyme.* In these right or left half embryos and larvæ the muscle and mesenchyme cells are present on one side of the notochord; here they occupy their normal positions, the muscle cells giving rise to three rows of cells along the lateral border of the notochord and the mesenchyme forming a group of small cells anterior to the muscle rows. (Figs. 32, 33, 36.) In later stages the muscle cells slowly extend over to the side of the tail on which they were originally lacking; this takes place especially at the hinder end of the tail, the overgrowth taking place around the end of the notochord and over its ventral side. In this way the right or left half embryo or larva tends to become complete, but I have never seen a case in which three rows of muscle

cells were found on both sides of the notochord. Indeed, I am not at all sure that this extension of the muscle cells around the end of the notochord is accompanied by any increase whatever in the number of muscle cells or in the number of rows of cells. The latest stage in which I can positively identify the three rows of muscle cells is shown in Fig. 36. In this larva the muscle rows lie nearer the ventral side than in normal larvæ (see Fig. 12), and they are evidently extending over the ventral surface toward the opposite side. In later stages the muscle cells become much elongated, but I have not been able to determine the number of rows present. I have found it still more difficult to decide whether the trunk mesenchyme ever extends over to the side on which it was originally lacking, but I believe that this takes place only to a limited extent, if at all, and that Chabry was right when he affirmed that only one atrial invagination is formed in these right or left half embryos.

2. *Three-Quarter Embryos (Figs. 34–35).*

In connection with the right or left half embryos I shall here consider three-quarter embryos, which, of course, include the whole of the right or left half. Two such embryos are shown in Figs. 34 and 35. In the former the left anterior quadrant was killed in the 8-cell stage; in the latter the right posterior quadrant in the 4-cell stage. The embryo in which the cells of the anterior quadrants were uninjured (Fig. 35) is perfectly normal in its anterior half; its posterior half, however, lacks those parts which would have developed from the cell which was injured. This embryo is younger than the one shown in Fig. 34 and no sense spots are present, but the sense vesicle is closing in a normal manner. This figure well shows that a part of the trunk mesenchyme is derived from the anterior quadrants, and indeed from the pair of cells $A^{7.8}$, Fig. 6, while a portion of it comes from the posterior quadrants, as may be seen by comparing the right and left sides of Fig. 35. The muscle cells are entirely lacking on the right side, the substance which would have formed them being located in the injured cell B^3; they are shown growing around the end of the notochord as in the half embryo shown in Fig. 33. The notochord and nerve tube are apparently full sized, which is explained by the fact that they come from the anterior quadrants, but owing to the lack of the right side of the tail they are somewhat distorted in form.



RIGHT OR LEFT HALF LARVAE. FIXED AND STAINED MATERIAL.

Figs. 37–40. Four half larvæ from eggs which were spurted in the 2-cell or 4-cell stage and fixed 22 hours later. These larvæ are still within the egg membranes though at a corresponding age normal larvæ are undergoing metamorphosis.

Fig. 37. Right half larva, ventral view. The tail which is elongated is turned down toward the dorsal side; the sense vesicle also lies on the dorsal side and is here seen through the embryo. The muscle cells are chiefly on one side of the notochord but have grown over to the other side at the posterior end.

Fig. 38. Left half larva, dorsal view. The neural plate with sense spots is partly covered by the end of the tail. The mesenchyme is found only on the left side.

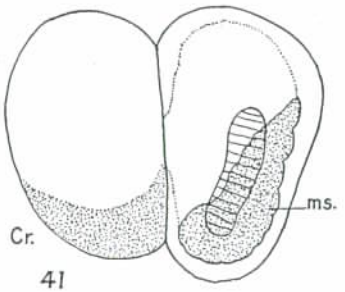
Fig. 39. Left half larva from the left side. The neural plate is folded so as to form a nearly closed sense vesicle, in which are two sense spots.

Fig. 40. Left half larva viewed from the left side. The neural plate is partially closed, but is abnormal in form. In all of these larvæ the neural plate lies on the concave side.

RIGHT OR LEFT HALF LARVAE DRAWN FROM LIVING SPECIMENS FROM 12 HOURS (FIGS. 41, 42) TO 20 HOURS (FIGS. 45, 46) AFTER THE INJURY OF ONE OF THE FIRST TWO BLASTOMERES.

Fig. 41. Posterior-dorsal view. Fig. 42. Same embryo, posterior ventral view. In both these figures the muscle cells are found chiefly on one side of the notochord, but they have grown over to the opposite side at the end of the tail. Fig. 43. Right half larva from right side. Fig. 44. Left half larva from left dorsal side. The yellow crescent on the injured blastomere apparently occupies different positions with respect to the larva in these two figures, but it is by no means certain that the convex side of the larva is morphologically the same in the two figures.

Figs. 45, 46. Two views of one and the same left half larva. Fig. 45, from the dorsal side; Fig. 46, from the left side, showing two sense spots on the dorsal and two on the ventral sides. The neural plate is continuous between these spots on the side next to the injured blastomere.



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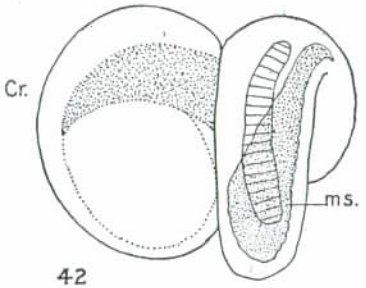

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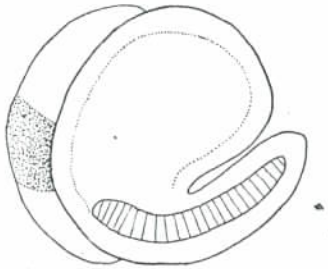
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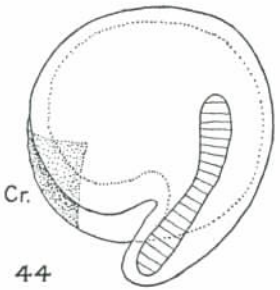

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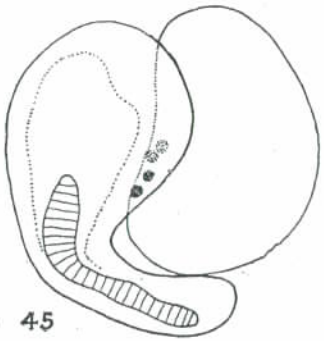
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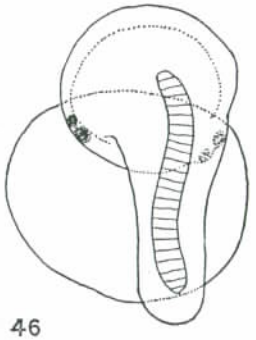
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In Fig. 34 the left anterior quadrant was killed and the posterior portion of this embryo is normal save only for the fact that the notochord and nerve tube are smaller than usual, which is explained by the fact that the substance of these organs is derived from the anterior quadrants; three rows of muscle cells are found on both sides of the tail. The anterior half of this embryo, on the other hand, is quite defective; the neural plate is irregularly folded and has not formed a sense vesicle, although sense spots are present.

I have seen and studied many three-quarter embryos similar to those shown in Figs. 34 and 35 and they all show, as do the right and left half embryos, that where part of the substance which would normally form an organ is destroyed the organ which develops is defective, whereas if all or any organ-forming substance is lacking the organ to which it would normally give rise is also lacking.

So far as I have observed these partial larvæ never escape from the egg membrane, and in this my observations accord with those of Chabry and Driesch, and although I have kept them alive until a period after the normal larvæ have undergone metamorphosis I have never observed this transformation in them.

In conclusion then I find that the cleavage and gastrulation of these half or three-quarter embryos is partial and the resulting larva incomplete although the notochord is well formed and there is a tendency on the part of some of the cells to grow over and close up the open side of the larva. However, this regulation never leads to the formation of a complete larva; the neural plate may close, but it forms an abnormal sense vesicle; at the end of the tail the muscle cells extend over toward the injured side, but they do not form three rows of cells on each side of the notochord as in the normal larva; the mesenchyme likewise does not develop along the injured side and it is probable that only one atrial invagination is formed.

Furthermore *not a single cleavage cell nor any one of the oöplasmic substances ever gives rise to parts or organs which it would not normally produce; the notochord, for example, invariably comes from the chorda cells, the sense vesicle from the neural plate cells and both these structures from the material of the gray crescent; the muscles always come from the muscle cells and these from the substance of the yellow crescent; the ectoderm, from the ectoderm cells and ultimately from the clear protoplasm; the endoderm, from the endoderm cells and these from the deep gray material of the egg.* In spite therefore of the regulation which is apparent in the closing of the open side of the embryo, and in the formation of a whole notochord and of an imperfect sense vesicle, the various oöplasmic substances of the unsegmented egg and of the different blastomeres are not totipotent but each shows in these experiments, as well as in normal development, that it is differentiated to give rise to one, and only one, particular kind of tissue.

3. *Anterior Half Embryos (Figs. 47–52).*

The anterior and posterior half embryos show even more clearly than do the lateral ones the mosaic character of the development of these eggs. When the posterior half of an egg is killed in the 4-cell or 8-cell stage the anterior half continues to develop as if the posterior half were still living. The cleavage is in all respects like that of the anterior half of a normal egg; the gastrulation is essentially the same, but the later development is modified in many important particulars.

Figs. 47 and 48 are ventral and dorsal views, respectively, of one and the same living embryo of the 76-cell stage, in which the posterior dorsal cells, $B^{4.1}$, containing the yellow crescent, were killed in the 8-cell stage. None of the cells of the ventral hemisphere were injured and consequently the cleavage of these cells is quite normal; thirty-two ectoderm cells are present, all of which have entirely normal positions, shapes and sizes. (*cf.* Figs. 5 and 47.) The anterior half of the dorsal hemisphere is also entirely normal (*cf.* Figs. 6 and 48); eight chorda cells are shown forming an arc which bounds anteriorly the six endoderm cells and which is flanked on each side by the anterior mesenchyme cell, $A^{7.8}$. The number, size and position of each and all of these cells is the exact counterpart of what is found in the normal embryo, and, although the outlines of the neural plate cells were so indistinct in the living specimen from which this figure was made that I could not draw them, there is every reason to suppose that these cells like all the others in this embryo conform to the normal type.

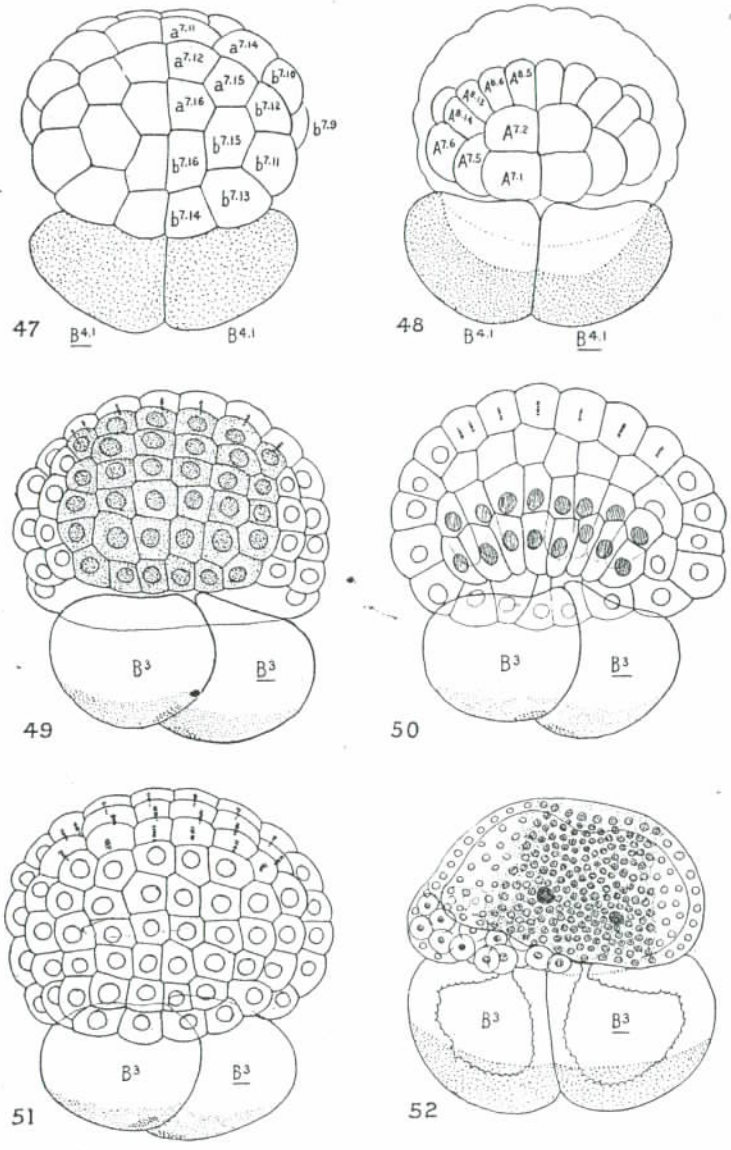
In the posterior half of the dorsal hemisphere all the parts which would have developed from the cells $B^{4.1}$ and $B^{4.1}$ are entirely lacking; there are neither mesenchyme, caudal endoderm,

ANTERIOR HALF AND THREE-QUARTER EMBRYOS; 76 CELLS TO METAMORPHOSIS.

Figs. 47, 48. Anterior-ventral three-quarter embryo of the 76-cell stage (*v.* Figs. 5 and 6); the dorsal posterior cells $B^{4.1}$, containing all of the yellow crescent, were killed in the 8-cell stage. The ventral ectoderm cells (Fig. 47) are quite normal both in position and number (*cf.* Figs. 5 and 47); the anterior dorsal cells are also normal, but the posterior dorsal cells (muscle, mesenchyme and caudal endoderm) are entirely lacking. (*cf.* Figs. 6 and 48.)

Figs. 49–51. Three views of one and the same anterior half embryo of about the 250-cell stage; spurted in the 4-cell stage and fixed 2 hours later. Fig. 49. Dorsal view, superficial focus, showing the neural plate. Fig. 50. Dorsal view, deeper focus, showing two rows of chorda cells besides several ectoderm and endoderm cells. Fig. 51. Dorsal view, still deeper focus, showing the cells of the ventral ectoderm.

Fig. 52. Anterior half embryo, dorsal view. Spurted in the 4-cell stage, fixed 22 hours later. The yellow crescent is plainly visible in the injured cells. Sense spots are present but the neural plate never forms a tube. The chorda cells lie in a heap at the left side. There is no trace of muscle substance or of a tail in this anterior half embryo. This embryo is from the same experiment as Figs. 37–40; normal larvæ of this stage are undergoing metamorphosis.



nor muscle cells. Unfortunately this particular embryo was not followed through the various stages of development until it gave rise to a larva and none of the older stages which I have studied have shown precisely this type of injury, *i. e.*, the destruction of the yellow crescent without injury to the ectoderm cells of the posterior half.

In many other cases which I have seen all of the posterior half of the egg was injured in the 4-cell stage. I have followed the development of the surviving anterior halves of such eggs as late as the stage of the metamorphosis of the normal larvæ; the development of such blastomeres is always partial. Figs. 49, 50 and 51 represent three views of one and the same anterior half embryo of about the 250-cell stage; in all the figures the embryo is viewed from the dorsal side, but in Fig. 49 the focus is high and only the ectoderm and neural plate cells of the dorsal surface are shown; Fig. 50 is a median optical section showing chorda and endoderm cells surrounded on the anterior side by ectoderm; Fig. 51 represents the ectoderm of the ventral surface which is visible at a deep focus. This half embryo is exactly like the anterior half of a normal one in the formation of the neural plate, the chorda plate, the general ectoderm and gastral endoderm, in the overgrowth of the dorsal lip of the blastopore, even in the position, shape and size of the individual cells. (*cf.* Figs. 9 and 10.)

Finally in Fig. 52 there is represented an anterior half embryo 22 hours after the posterior cells were killed, and at a stage when normal larvæ of corresponding age have already undergone metamorphosis. The ectoderm has not yet inclosed the embryo on the side next the injured cells, and this rarely happens in anterior or posterior half embryos. The neural plate has not rolled up nor invaginated to form a tube, though it is slightly depressed along its median line; two sense spots are present though there is no sense vesicle. The large rounded chorda cells are irregularly scattered along the posterior border of the embryo, where they project beyond the ectoderm; they never form a notochord. There is no trace of yellow crescent substance nor of muscle cells in these anterior larvæ and no indication whatever of a tail. They are, therefore, altogether unlike the normal larvæ and they afford complete and convincing evidence that the anterior blastomeres of the ascidian egg are not totipotent but rather that the development is a mosaic work.

4. *Posterior Half Embryos (Figs. 53–58).*

All that has been said of the mosaic-like development of the anterior half of the egg is equally true of the posterior half. The cleavage progresses in normal fashion up to the time of the closure of the blastopore. Figs. 53 and 54 represent posterior half embryos of the 32-cell and 76-cell stages, respectively. The former is entirely normal and the latter is normal in all respects save that a single pair of cells, $B^{8.6}$, is larger than in the normal embryo. The clear, the yellow and the gray substances of the egg are distributed exactly as in the posterior half of a normal embryo. The clear ectoderm cells lie on the ventral side and only two of them appear in the dorsal view shown in Fig. 54 (the two clear cells at the posterior pole). In Fig. 53 the gray endoplasm is contained in two cells ($B^{6.1}$) and in Fig. 54, in four ($B^{7.1}$, $B^{7.2}$); these cells give rise to the strand of caudal endoderm. The yellow crescent consists at the 32-cell stage of a single arc of yellow cells (Fig. 53) which then, by division, become a double arc of fourteen cells (Fig. 54); the inner arc consists of eight mesenchyme cells and the outer of six muscle cells. In all these respects these posterior half embryos are entirely like the posterior half of a normal embryo.

But while the pregastrular stages of these posterior half embryos are like the normal, the gastrulæ and later stages show many interesting modifications. Figs. 55, 56, 57 are three views of one and the same posterior half embryo, the normal embryos of the same stage being young tadpoles like Fig. 11. In all of these figures the embryo is viewed from the dorsal side; Fig. 55 shows the ectoderm cells which cover the dorsal surface; Fig. 56, the muscle cells which lie below the ectoderm on the dorsal side; Fig. 57 is an optical section at a still deeper level showing the caudal endoderm and mesenchyme. Fig. 58 is another posterior half embryo of similar age seen from the ventral side, showing the yellow mesoderm cells on each side of the caudal endoderm.

The gastrulation occurs between the stages shown in Figs. 54 and 55. The caudal endoderm and the surrounding arc of mesenchyme, shown in Fig. 54, invaginates; the muscle cells come to lie above (dorsal to) the mesenchyme cells and finally the latter are overgrown by the ectoderm in the manner shown in Fig. 8. In normal embryos the posterior part of the blastopore is closed chiefly by the growth of the anterior lip; in the latter stages of

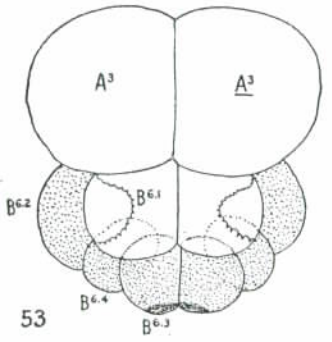
POSTERIOR HALF EMBRYOS; 32 CELLS TO TADPOLE STAGE. FIXED AND STAINED PREPARATIONS.

Fig. 53. Posterior half of 32-cell stage, dorsal view. The cleavage is altogether normal. Spurted in the 4-cell stage, fixed 1 hour later.

Fig. 54. Posterior half of 76-cell stage (*cf.* Fig. 6); spurted in the 4-cell stage, fixed 2 hours later. Two rows of yellow crescent cells are present, the inner being mesenchyme, the outer muscle cells; the anterior pair of mesenchyme cells ($B^{8.6}$) are larger than normal. There are two pairs of caudal endoderm cells ($B^{7.1}$ and $B^{7.2}$). A pair of ventral ectoderm cells is visible in the midline behind.

Figs. 55–57. Three views of one and the same embryo; spurted in the 4-cell stage, fixed 4 hours later, normal embryos being in the stage represented by Fig. 11. Fig. 55. Dorsal view of the superficial ectoderm. The notch in front represents the notch in the ventral lip of the blastopore. Fig. 56. Same view, deeper focus, showing the muscle cells beneath the ectoderm; these cells are continuous from side to side, there being no chorda in the midline. Fig. 57. Same view, still deeper focus, showing the double row of ventral endoderm cells in the midline, and on each side of this a mass of mesenchyme cells.

Fig. 58. Ventral view of posterior half embryo of the same stage as the preceding, showing the muscle and mesenchyme cells beneath the ectoderm and on each side of the strand of ventral endoderm.



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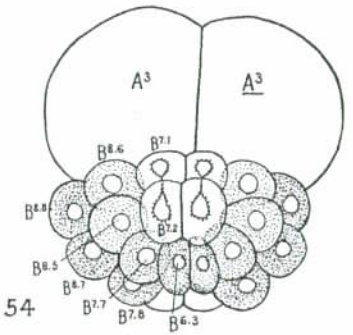

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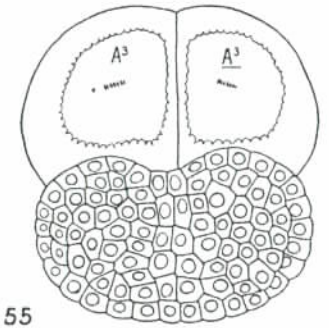

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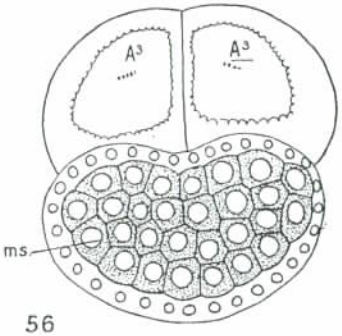

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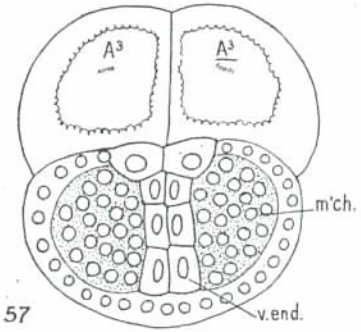

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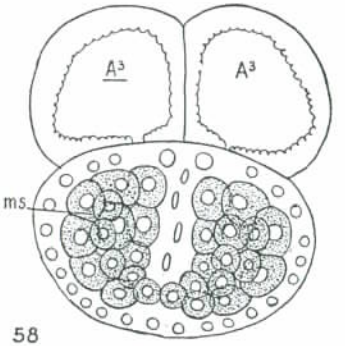

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gastrulation a blastopore groove is left in the posterior half of the embryo, on each side of which lie the muscle cells. (Fig. 9.) By the continued growth of the anterior lip this groove is shoved to the posterior end of the embryo and the rows of muscle cells are tilted up from an antero-posterior to a vertical position. Later, when the notochord is formed, the muscle cells come to lie alongside of it, thus forming the three rows of muscle cells on each side. Finally the ectoderm of the posterior lip of the blastopore, which has, up to this stage, formed a notch at the end of the blastopore groove, grows forward and reduces this groove to a minute pore.

Owing to the absence of the anterior lip of the blastopore, and of the notochord and the neural plate, the later stages in the development of these posterior half embryos is much altered. In the first place the blastopore groove and the muscle cells are not pushed to the posterior end of the embryo. Then the muscle cells on each side of the blastopore groove are not kept apart by the notochord but come into contact forming a continuous layer of muscle cells across the dorsal side. (Fig. 56.) The blastopore groove, therefore, disappears by the fusion of the lateral lips of the groove and the ectoderm cells grow over the whole dorsal surface; the only trace of the blastopore groove which is left is a slight notch in the anterior border of the embryo. (Figs. 55, 56.) The ectoderm never entirely incloses the posterior half embryo on the side next the injured cells, but the endoderm here comes to the surface as shown in Figs. 57 and 58.

No trace of notochord, neural plate nor sense spots ever appears in these posterior half embryos, and what is more remarkable a tail is never formed but the embryo always remains rounded in form, as shown in Figs. 55–58. It is quite evident that the elongation of the tail of the normal larva, together with the elongation of the individual muscle cells and perhaps also the arrangement of these cells in three rows on each side, is dependent upon the presence and elongation of the notochord. Perhaps one reason why a normal notochord is never formed in the anterior half embryo is due to the fact that the ectoderm does not completely inclose the embryo, so that the chorda cells in their growth crowd out of the open side and hence become free and scattered.

In conclusion, the study of anterior or posterior half embryos establishes in a most convincing manner the fact that the development of individual blastomeres of the ascidian egg is a mosaic work.

These blastomeres give rise only to those tissues and parts of an embryo which would come from them normally. Nothing even remotely resembling a complete normal larva is ever produced from the anterior or posterior quadrants of the egg.

5. *Quarter Embryos (Figs. 59–70).*

The development of individual blastomeres of the 4-cell stage furnishes additional confirmation of the mosaic theory as applied to ascidian eggs; in every instance individual blastomeres give rise only to those parts or organs which they would produce in normal embryos. Quarter embryos generally show more abnormalities and variations than half embryos,—probably owing to the more severe injury which they have suffered, which often affects the surviving quarter of the egg.

The cleavage of these quarter eggs is normal in every detail, save that the position of the cells is sometimes slightly altered; the rhythm of cleavage and the size and quality of the cells is the same as in the corresponding quarter of a normal egg. In Fig. 59, which corresponds to the 16-cell stage of the normal egg, each of the surviving quadrants has divided twice; in Fig. 60 the left posterior quadrant of a 44-cell stage is shown and in both of these figures the size, quality and position of the cells as well as the rhythm of division and the distribution of the different oöplasmic substances is entirely normal. Fig. 61, which is the right anterior quadrant of the 76-cell stage, is normal in every respect, save for the position of the endoderm cells which are here displaced toward the first cleavage plane. The mesoderm cells in the right posterior quadrant, shown in Fig. 62, are not normal in position; the two caudal endoderm cells (lying next the first cleavage plane) are, however, normal and the ectoderm cells are normal save that they show a tendency to grow inward at the first and second cleavage furrows and thus surround the embryo. In particular, attention should be directed to the yellow crescent and caudal endoderm cells in Fig. 60, and to the neural plate and chorda arcs in Fig. 61, which are similar in every respect to the quarter of a normal embryo at these stages.

I have already commented upon the fact that the quarter embryo shown in Fig. 63 is a "false gastrula" since the invaginated cells are ectodermal, probably neural plate cells, while the larger endo-

QUARTER EMBRYOS; 16 CELLS TO YOUNG TADPOLE STAGE. FIXED AND STAINED PREPARATIONS.

Fig. 59. Left anterior and right posterior (diagonal) quarter embryos of the 16-cell stage, ventral view.

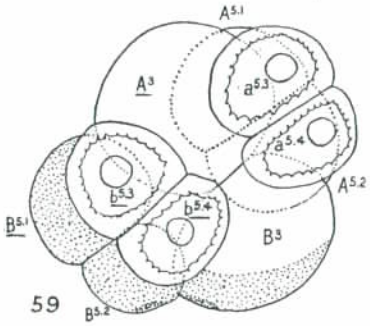
Fig. 60. Left posterior quarter embryo of the 44-cell stage, posterior view.

Fig. 61. Right anterior quarter embryo of the 76-cell stage, dorsal view, showing the neural plate and chorda cells of the right side.

Fig. 62. Right posterior quarter embryo of about the 180-cell stage, dorsal view (*cf.* Figs. 7, 8); spurted in the 4-cell stage, fixed 2½ hours later, showing 6 muscle and 2 caudal endoderm cells.

Fig. 63. Left anterior quarter embryo, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. An invagination of the ectoderm cells has the appearance of a gastrula, but is probably the invagination of the neural plate.

Fig. 64. Left anterior and right posterior (diagonal) quarter embryos, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. Muscle cells are found only in the posterior quarter.



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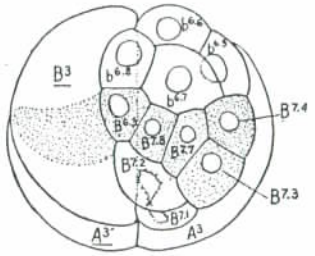

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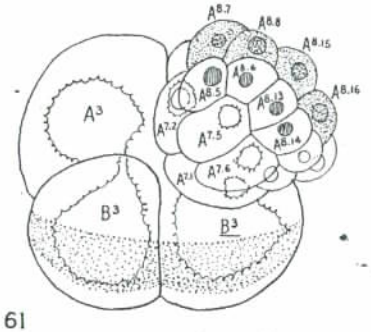

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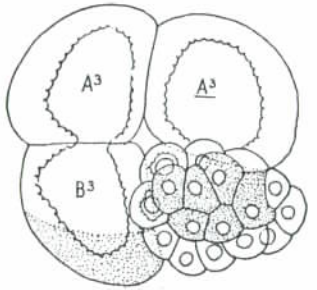

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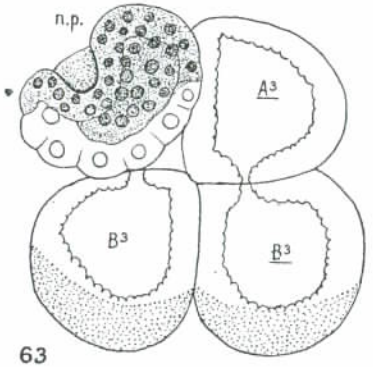

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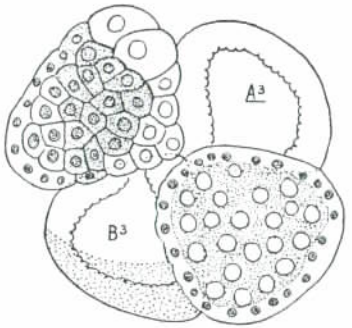

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QUARTER EMBRYOS; YOUNG TADPOLE TO METAMORPHOSIS STAGES. FIXED AND STAINED PREPARATIONS.

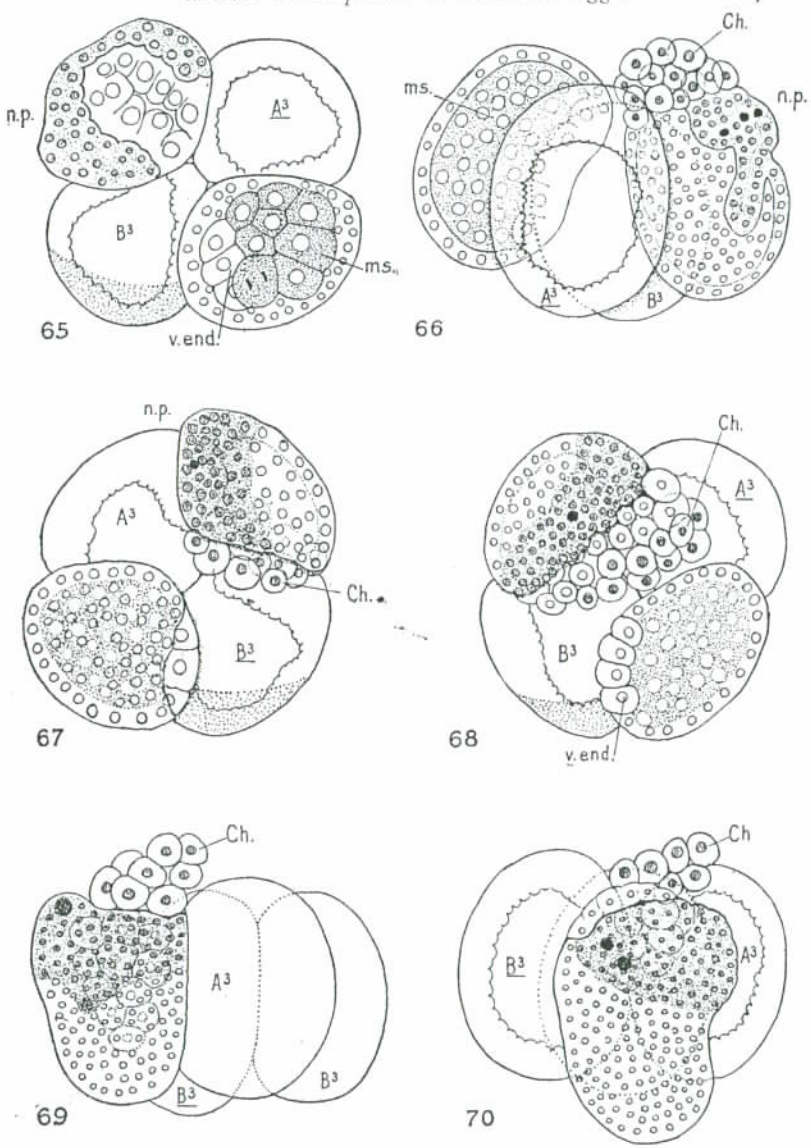
Fig. 65. Left anterior and right posterior (diagonal) quarter embryos, dorsal view; spurted in the 4-cell stage, fixed 5 hours later. The anterior quarter shows thickened ectoderm cells, probably neural plate, around the endoderm cells; in the posterior quarter are 8 muscle and 3 caudal endoderm cells.

Fig. 66. Left anterior and right posterior (diagonal) quarter embryos from the right anterior side, the dorsal pole being above; spurted in the 4-cell stage, fixed 22 hours later. In the posterior quarter the muscle and mesenchyme cells form a solid mass; in the anterior quarter the chorda cells project freely over the dorsal surface and the neural plate is partially infolded and contains three sense spots.

Fig. 67. Right anterior and left posterior (diagonal) quarter embryos, dorsal view; spurted in 4-cell stage, fixed 22 hours later.

Fig. 68. Left anterior and right posterior (diagonal) quarter embryos, dorsal view; spurted in 4-cell stage, fixed 22 hours later. In this and the preceding figure the chorda cells (Ch.), neural plate (n. p.) and sense spots are found only in the anterior quarters; the muscle, mesenchyme and caudal endoderm cells, only in the posterior quarters.

Figs. 69, 70. Right anterior quarter embryos, dorsal side above; spurted in 4-cell stage, fixed 12 hours later. These embryos show free chorda cells, neural plate and sense spots, but not a trace of muscle cells.



derm cells remain on the rounded surface of the embryo. I have not observed in detail the process of gastrulation in any of these quarter embryos, but it is evident that there is no considerable gastrula cavity and that the endoderm cells are chiefly overgrown by the ectoderm, as shown in Fig. 65. Ultimately the endoderm and mesoderm are largely overgrown, though in this case, as in the half embryos, the ectoderm does not entirely inclose the embryo on the side next to the injured cells and through the opening thus left some of the endoderm cells may protrude.

Although the localization of oöplasmic substances and of organ bases is usually the same as in the quarter of an entire embryo, in some cases there are dislocations of these substances and bases which are probably due to injury of the surviving quarter. Thus in the left anterior quarter, shown in Fig. 64, large endoderm cells lie at the surface next to the first cleavage plane; in the same quadrant of another egg shown in Fig. 65 the neural plate cells lie at the periphery of the quadrant and chiefly on the left side, instead of along the median plane as in normal embryos. I have seen many other instances of such dislocations but they are all of such nature that they can be interpreted as due to slight injury to the surviving blastomeres. In not a single instance are parts derived from a blastomere which would normally have come from another cell.

The anterior quarter embryos are always recognizable by the presence of the neural plate and, in later stages, of the sense spots. The neural plate usually remains at the surface and is not infolded, but in some cases it is invaginated through at least a portion of its area, though a sense vesicle is not formed. (Figs. 63, 66.) In all later stages one or more sense spots appear in the plate. (Figs. 66–70.) The neural plate always lies along the dorsal side of the embryo, though it may be shifted more or less from the median plane. (Figs. 65–70.) The chorda cells are found exclusively in the anterior quadrants and in later stages they protrude to the exterior along the injured side where they are found as scattered cells in the perivitelline space. (Figs. 66–70.) In no case, save one, have I seen any indication that these cells form a rod-shaped notochord, and this case (Fig. 72) was that of a living embryo in which it is possible that the notochord-like structure was really composed of gastral endoderm and hence not a true notochord at all. It is evident that the chorda cells are unable to give rise to a notochord when once they have escaped and have become free, a certain amount of compression being necessary to bring about the characteristic interdigitation which leads to the formation of a rod-shaped notochord.[1]

The posterior quadrants can be distinguished in all eggs at all stages by the presence of the yellow crescent substance or cells. In early stages, as I have shown, these crescent cells are normal in position and character; in later stages the yellow cells fill the whole interior of the embryo. When once these cells have been inclosed by the ectoderm I have been unable to recognize any constancy in their position and arrangement. As in the posterior half embryos, a tail is never formed in these posterior quarter embryos and the muscle cells are never elongated, both these features evidently depending upon the presence of a notochord. The caudal endoderm cells are found in most, if not all, of these posterior quarter embryos as a single row of yolk laden cells which lie along the first cleavage plane (Figs. 65–68), the position which they normally occupy.

These quarter embryos show in the most unmistakable manner that the development is strictly partial, and that an individual blastomere never gives rise to parts which it would not produce in the entire embryo. Among the hundreds of quarter embryos which I have studied both in the living condition and as stained and mounted preparations I have never seen a single one which even remotely resembled a normal larva.

6. *Eighth or Sixteenth Embryos (Figs. 71–76).*

When eggs are spurted or shaken in the 8-cell and 16-cell stages a great variety of abnormal forms are produced, a few of which are shown in Figs. 71 and 73–76. Without exception, however, the same principles apply here as in the case of half and quarter embryos, viz: a given blastomere or group of blastomeres produces only those parts of an embryo or larva which would develop from it under normal conditions. Fig. 71 represents an embryo derived from the dorsal anterior eighth of an egg (the cell $A^{4.1}$) 14 hours after the injury. Normally this eighth gives rise to neural plate, chorda, gastral endoderm, and a small amount of

[1]Chabry, however, figures (his Fig. 18) a partial embryo with a rod-shaped notochord lying outside the embryo in the perivitelline space.

PARTIAL EMBRYOS FROM ISOLATED BLASTOMERES OF 8-CELL OR 16-CELL STAGES. DRAWN FROM LIVING SPECIMENS.

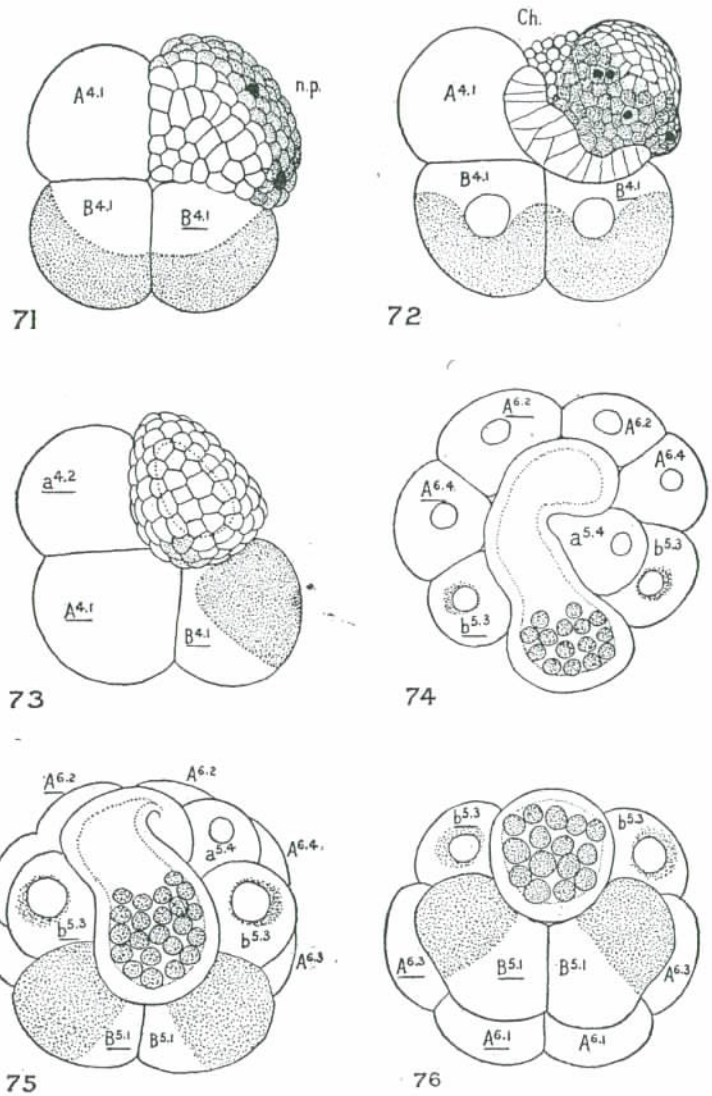
Fig. 71. Right anterior dorsal eighth embryo, 14 hours after injury, showing endoderm, chorda, and neural plate cells with sense spots.

Fig. 72. Right anterior quarter embryo, 14 hours after injury, showing chorda, neural plate, and sense spots.

Fig. 73. Posterior ventral quarter embryo derived from the cells $b^{4.2}$, $b'^{4.2}$ and containing no endoderm and only a small amount of yellow protoplasm which was derived from the perinuclear plasm of the cells $b^{4.2}$, Fig. 13.

Fig. 74–76. Three views of a partial embryo derived from 7 cells of the 20-cell stage, viz: 2 ($B^{4.2}$), 2 ($b^{5.4}$), 1 ($a^{5.4}$), 2 ($a^{5.3}$). (*cf.* Figs. 3 and 4.) The embryo consists of an outer layer of clear ectoderm and of a mass of yellow mesenchyme cells derived from the cells $B^{5.2}$, but it is wholly without endoderm.

Fig. 74, Ventral view; Fig. 75, Posterior; Fig. 76, Postero-dorsal.



mesenchyme derived from the cell $A^{7.6}$. In the embryo shown in Fig. 71 the neural plate cells are clearly shown around the periphery of the figure and two of the cells contain sense spots. The chorda, endoderm, and mesenchyme cells are shown internal to the neural plate, but I am unable to distinguish in this embryo between these three kinds of cells; they are all more or less yolk-laden as in the normal egg. Owing probably to the fact that no ventral ectoderm cells are present the neural plate is not pushed up onto the dorsal face and there are no evidences of gastrulation, although normal embryos of a corresponding age have already reached the full larval development. That this failure to gastrulate is not due to the slower development of the egg fragments as compared with the entire egg is shown by the degree of histological differentiation of the neural plate and sense spots, the latter appearing normally only in the fully formed larvæ.

Fig. 72 is a quarter embryo of the same age as the preceding, derived from the cells $A^{4.1}$, $a^{4.2}$ of the right anterior quadrant. The ventral ectoderm cells have here pushed the neural plate cells up onto the dorsal face of the embryo, while the chorda cells (?) lie along the median and transverse furrows. Four sense spots are present in the neural plate.

Fig. 73 is also a quarter embryo of the same age as the preceding, derived from the two posterior ventral cells $b^{4.2}$, $b^{4.2}$. This embryo consists entirely of ectoderm which is arranged in a single layer of cells around a central cavity, the blastocoel. There has been no gastrulation and the embryo contains neither endoderm nor mesoderm. A few of the ectoderm cells next to the cell $A^{4.1}$ contain yellow pigment, exactly as in the normal embryo.

Figs. 74 to 76 are three views of one embryo, about 20 hours after the egg was spurted in the 20-cell stage. By the spurting all the cells were killed except seven from which this embryo has developed, viz: a pair of mesenchyme cells $B^{5.2}$, and five ectoderm cells, $b^{5.4}$, $b^{5.4}$, $a^{5.3}$, $a^{5.3}$ and $a^{5.4}$. (See Fig. 3.) This embryo consists entirely of an outer layer of clear ectoderm cells, inclosing at its posterior end a mass of small mesenchyme cells; it contains no endoderm. It is an interesting fact that the mesenchyme cells are here inclosed by the ectoderm, showing that some process in the nature of gastrulation must have taken place.

A great many other partial embryos, produced from one or more blastomeres of the 8, 16 or 32-cell stages, have been studied

but they all illustrate the principle that a blastomere never gives rise to any other structures than those which it would produce in a normal embryo.

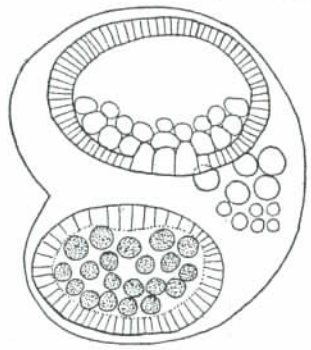
7. *Anterior and Posterior Half Gastrulæ (Figs. 77–82).*

In a recent publication Driesch ('03) has maintained that an alteration in the capacity for regulation occurs in the ascidian development between the early and the late gastrula stages. When the open cup-shaped gastrulæ of Phallusia were cut in two transversely into anterior and posterior halves, each of these halves developed into "einer vollständigen kleinen Appendicularie, welcher Organe niederer Bedeutung (Otolith, Augenfleck) eventuell fehlten." However, when the elongated gastrulæ were cut in two transversely a head developed from one piece and a tail from the other, "so deutlich und sharf begrenzt und ausgebildet, als habe man eine fertige Appendicularie scharf durchschnitten."

Considering the results which I have obtained on the development of the two anterior or two posterior cells of the 4-cell stage of Cynthia the conclusions of Driesch seemed most remarkable and I therefore undertook to repeat his experiments upon Cynthia. Gastrulæ of the stage shown in Fig. 8 were cut in two with a sharp knife made from a needle, under a Zeiss binocular dissecting microscope. With the power used the individual cells of the yellow crescent could be plainly seen and it was always easy to determine the exact boundary between the anterior and posterior halves. In every instance the section was made as close as possible to this boundary (second cleavage plane) and so as to leave all of the yellow cells in one of the pieces. Owing to the presence of the chorion the experiment was not an easy one to perform, since the chorion would frequently slip under the knife, or the egg move within the chorion. Nevertheless in one day I succeeded in cutting in two about thirty of these early gastrulæ; ten of these lived for twenty hours or longer after the operation, the others were too badly crushed to survive. Four of these which survived the operation are shown in Figs. 77–82, the drawings having been made from nineteen to twenty hours after the operation. Every one of these ten surviving embryos was a partial one and, although I was unable to determine their structure with the same amount of detail as in the case of stained and mounted preparations, it was

ANTERIOR AND POSTERIOR HALF GASTRULAE.

Figs. 77–82. Partial embryos derived from gastrulæ of the stage shown in Fig. 8, which were cut in two transversely so as to leave the whole of the yellow crescent in one half. The chorion is shown as a line around the embryos. Figs. 77, 78. Dorsal and ventral views respectively of one and the same embryo, drawn 19 hours after the operation. A mass of cellular débris lies between the two half embryos; the endoderm cells are chiefly contained in the anterior half, the mesenchyme and muscle cells are entirely confined to the posterior half. Neither half at all resembles a normal embryo or larva. Figs. 79, 80. Ventral and postero-dorsal views of another embryo, 19½ hours after the operation. The crescent of yellow cells is entirely confined to the posterior half and neither half resembles a normal larva. Fig. 81. Anterior and posterior half embryos 20 hours after the operation. Fig. 82. Posterior half embryo from the postero-dorsal side, 20 hours after the operation. The anterior half is degenerating and is shown only in dotted outline; the posterior half contains all of the yellow cells and practically no endoderm. At the stages represented by all these figures the normal embryos have already undergone their metamorphoses.



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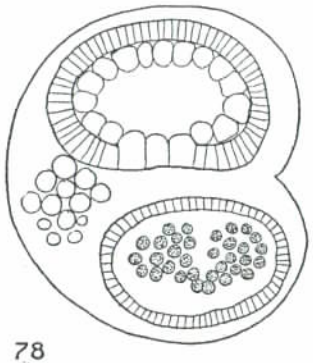

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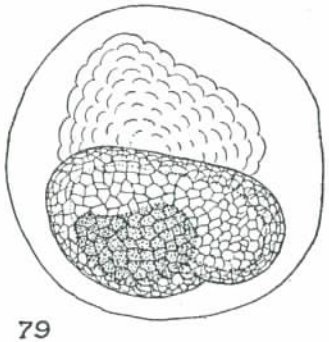

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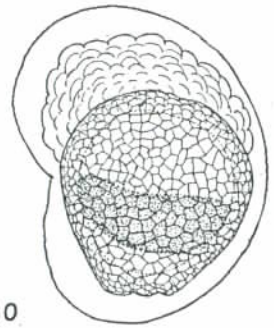

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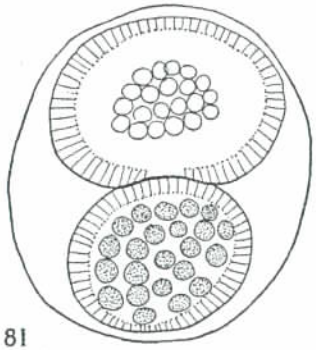

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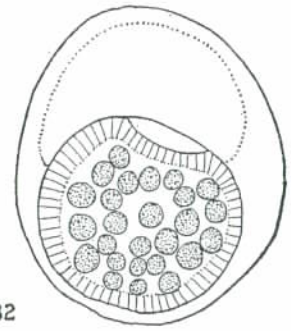

82

quite evident that not one of them resembled in any respect whatever a normal larva. In some cases both halves survived, as shown in Figs. 77–81, in other cases one half only survived. In all cases the surviving halves became rounded in form after the operation, the more seriously injured cells being crowded out of the embryo and forming a cellular mass of débris within the chorion. In every instance the surviving halves remained within the chorion, which was sometimes infolded as shown in Figs. 77–80. Each half was surrounded by a layer of clear ectoderm cells; the yellow cells were always found exclusively in the posterior half, the gray endoderm cells largely in the anterior half. Nothing resembling a notochord or neural tube ever developed in either half and no structure resembling a tail was ever formed. In fact these half embryos produced by cutting the early gastrulæ in two were altogether like the anterior and posterior half embryos which I have already described. (*cf.* Figs. 77–82 and Figs. 47–58.)

These results were so definite and conclusive that I did not continue the experiments and I regret now that I did not also cut gastrulæ in two along the median plane, though there is no reason to doubt that the results would be the same as in cases where one of the first two blastomeres is killed.

Comparing these results with those of Driesch, only one of two explanations is possible. Either Phallusia must differ most fundamentally from Cynthia, or Driesch must have mistaken the median for the transverse plane in these cup-shaped gastrulæ. That the former possibility is not probable is evidenced by the fact that the cell-lineage of all ascidians so far studied is essentially the same; furthermore my results as to the development of anterior and posterior halves of the egg of Cynthia are confirmed by my experiments on Molgula, as well as by Chabry's experiments on Ascidia aspersa. There is every reason to believe that what is true of these three genera is also true of Phallusia. On the other hand there are certain evidences that Driesch may have mistaken the transverse plane for the median; on p. 56 he says, "Aber auch an der Bechergastrula kann man die künftige Mediane und also auch die Hauptrichtungen senkrecht zu ihr unterschieden: es verlaufen nämlich die Zelltheilungsgrenzen des Ektoderms dieser Objecte so, dass sie gerade in der Medianen eine über die ganze Oberfläche fortgesetzte, nur sehr wenig gebrochene Einheitslinie bilden (S. z. B. Castle, Fig. 62, 71) welche ohne Weiteres

schon bei schwacher Vergrösserung kenntlich ist; schneidet man also in der Mitte und senkrecht zu dieser Linie, so zerlegt man auch die Bechergastrula in 'vorn' und 'hinten.'"

It is true that the median plane is marked out by a nearly straight line, though Castle's figures to which Driesch refers show this line between endoderm and not between ectoderm cells, but any one who has studied these embryos knows how difficult it is to determine the median plane in this way, especially in living material. Even in stained and mounted preparations it would not be a sure guide, much less could it be relied upon in the study of living gastrulæ. Whether the median plane appears as a straight line or not depends entirely upon whether that plane lies directly in the line of vision, and conversely some of the transverse planes of cleavage may appear as straight lines if they lie in the line of sight. Thus Fig. 7 shows several transverse rows of ectoderm cells which in the hinder part of the embryo are curved back in the middle and forward at the sides, but if the embryo were rotated forward so that the polar body were brought to the highest point these transverse rows would appear nearly straight.

I am convinced therefore that the half gastrulæ from which Driesch obtained apparently normal larvæ were right or left halves and not anterior and posterior ones as he supposed. Whether these larvæ were really normal, *i. e.*, whether they had the organs of both the right and left sides, cannot be determined from Driesch's figures or descriptions, since he seems to have considered that the only evidence required to show that a larva is complete is that it should have a head and a tail.

The fact that Driesch always obtained partial larvæ from the anterior and posterior halves of an elongated gastrula, where the chief axis is unmistakable, requires no comment.

IV. OTHER EXPERIMENTAL WORK ON THE ASCIDIAN EGG.

Chabry's ('87) contribution on the normal and teratological embryology of ascidians contains not only the most careful and complete experimental work which has ever been done on the ascidian egg but it is at the same time such an excellent analytical treatment of the normal development that it deserves to rank as an embryological classic. The experimental part of his paper was based upon an unusual knowledge of the normal and patho-

logical development of this species and it was carried out with a delicacy and precision of method which has never been surpassed. Add to this the fact that the work was undertaken with clear insight into the principal problems involved and at a time when almost no other work of this sort had ever been done[1] and its right to rank as one of the great works in experimental embryology seems assured. Considering these facts it is surprising that this work should have received so little attention and that it should have been so widely misunderstood or discredited.

Chabry's extensive experiments deal with right and left half embryos, anterior and posterior two-quarter embryos, and various forms of three-quarter, one-quarter and two-quarter diagonal embryos, and in all of these I find that my results are in the main in accord with his. The points in which my work is more detailed than his concern the presence and distribution of the various oöplasmic substances and the more accurate study of some of the later stages, made possible by the use of fixed and stained material. That the substance of the mesodermal crescent was seen by Chabry as early as the 32-cell stage is evident from his description of the mesoderm cells, which in Ascidia aspersa are greenish ("verdâtre") in color and which he recognized when only three were present on each side. Neither Driesch nor Crampton speak of having observed any of these oöplasmic substances and neither of them studied the later stages by means of fixed and stained material.

1. *Cleavage.*

Chabry showed that in rhythm of cleavage and in the size and character of the daughter cells the isolated blastomeres of Ascidia behave as if they were still part of the normal egg, while he described in great detail the changes which take place in the facets between cells. Crampton's conclusions are very similar; he found that "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." Driesch, on the other hand, found in Phallusia that there was no fixed relation between the

[1] See Roux, Ges. Abhand II, p. 958.

cleavage planes of the surviving half and the dead blastomere; that after the third cleavage the cells occupy very different positions from the normal (Tetraeder, Halbtetraeder); that divisions may be equal or unequal at the fourth cleavage, and finally that the cleavage could not be regarded as partial ("halb") nor entire ("ganz") but "regellos-solid." The evidence which Driesch brings in support of this conclusion is of little value since it is plain that he was unable to orient these cleavage forms and did not know from what part of the original egg they came nor from what pole they were viewed. My observations on the cleavage of isolated uninjured blastomeres of the egg of Cynthia confirm and extend the conclusions of Chabry and Crampton that the cleavage of such blastomeres is unaltered save for slight changes in the direction of some of the divisions; they are opposed to the conclusions of Driesch that the cleavage of such blastomeres is inconstant and irregular.

2. *Gastrula.*

Chabry figures four gastrulæ from isolated blastomeres, viz: his Figs. 108, 114, 129 and 130. O. Hertwig, who copies Fig. 129 in his book, "Die Zelle" ('98), says that it is a normal typical gastrula. Similarly Korschelt and Heider, who also copy this figure in their text-book ('02), affirm that it is a normal small gastrula. However, these authors bring no particle of evidence to the support of this bare assertion; Chabry himself nowhere says that any of the gastrulæ figured by him are normal and the figures themselves do not show that such is the case. On the other hand I can positively affirm that a normal entire gastrula is never formed from an isolated blastomere of the egg of Cynthia. In the absence of any evidence in favor of Hertwig's and Korschelt and Heider's interpretation and in the face of this positive evidence against it I think it may safely be assumed that Chabry's figures are not those of normal typical gastrulæ. Crampton expressly says that he did not carefully observe the process of gastrulation in the embryos derived from isolated blastomeres of the Molgula egg, but Driesch says that the process of gastrulation may be easily observed in Phallusia, that a typical ascidian gastrula is formed and that the closure of the blastopore takes place in the normal manner. "Alles sind verkleinerte Aehnlichkeitsbilder der Processe an normalen Eiern, welche stets vergleichen wurden."

However, it is quite evident from the observations of Van Beneden and Julin, Chabry, Castle and many others that something more than a mere invagination is necessary to constitute a normal gastrula. The ascidian gastrula is bilaterally symmetrical and its anterior and posterior portions are very unlike; furthermore all the principal organs of the larva are here represented by cells of peculiar structure and localization. In order to determine whether a gastrula is normal or not all of these features have to be considered, and this Driesch has not done.

3. *Larva.*

It is somewhat surprising that doubt should have been expressed as to whether Chabry obtained half embryos or whole embryos of half size from one-half of the ascidian egg. He again and again declares that lesion of a single cell up to the 16-cell and probably up to the 32-cell stage always causes a "hemiterie," or monster. (Chabry, pp. 246, 249, 250, 257, 258, 261, etc.) He even enters into a calculation of the number of kinds of monsters which may be produced by injuries to the cleavage cells. He says that if at the 8-cell stage each cell is capable of four different kinds of modification (certainly less than the reality), the number of modalities of this stage is 4^8 ($= 65536$) of which only one is normal. In this way there arises that "admirable and infinite variety of monsters" to which he repeatedly refers. He says expressly, p. 289, "De là on tire aisément la conclusion (que je ne crois valable que pour l'Ascidie et les animaux, dont les blastomères sont différenciés de bonne heure), que chaque blastomère contient en puissance certaines parties dont sa mort entraîne la perte irrémédiable et que les différentes parties de l'animal sont préformées dans les différentes parties de l'oeuf." Again on p. 299 he says, "On ne saurait donc conclure avec sécurité de l'oeuf d'Ascidie à celui des autres animaux, mais, en ce qui concerne celui-ci, il est exact de dire qu'il se comporte comme s'il contenait en puissance un seul adulte déterminé et que chaque partie de l'oeuf contint une partie de cet adulte." This same conclusion is repeated again and again so that as Barfurth ('93) and Driesch ('95) have said there can be no question as to what Chabry believed that his observations and experiments proved.

The statements of Driesch and Crampton are even more positive and explicit that whole larvæ are formed from any one or more of the first four blastomeres. Driesch (p. 405 in summarizing his results uses, in part, the very words of his conclusions regarding the value of the cleavage cells in the echinoderm egg: "Aus isolirt überlebenden Blastomeren des Ascidieneies entwickelt sich nicht ein halber (resp. viertel, drei viertel) rechter oder linker (resp. vorderer oder hinterer) Embryo, sondern stets ein ganzer von halber Grosse, dem allerdings (meist) gewisse Organe von minderen Bedeutung (Otolith, ein Haftorgan) fehlen." Crampton neither figures nor describes the larvæ obtained from isolated blastomeres of Molgu'a, but he says, p. 55, "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."

Chabry first discovered that larvæ from one of the first two blastomeres were superficially like normal larvæ in that they had head and tail, notochord, neural plate and sense spots, but he showed that they also lacked the organs distinctive of the missing side, viz: one papilla, one or more sense spots and one atrial invagination. It is surprising therefore that neither Driesch nor Crampton undertook to prove that the larvæ obtained by them from one of the first two blastomeres were really complete. One looks in vain in their papers for any evidence that the organs characteristic of that side which would have developed from the dead half (muscles, mesenchyme, papilla, atrial invagination) are present in the surviving half.

Chabry further showed that the type of embryo derived from the anterior or posterior two-quarters of the egg was very unlike that derived from the right or left two-quarters, while the one-quarter embryos were still more unlike the normal; in each of these cases he found that the development was strictly partial, only those parts arising from a blastomere which would develop from it in the normal embryo. In the face of these conclusions of Chabry's neither Driesch nor Crampton advance any evidence in favor of their claim that the anterior and posterior quadrants of the egg as well as the right or left may give rise to a larva. Chabry's figures and descriptions show plainly what my work proves that nothing even remotely resembling a normal larva is ever pro-

duced from any portion of an egg which does not include the whole of the right or left half. In my opinion Driesch and Crampton have not studied nor taken any account of anterior or posterior half embryos, but only of right or left ones. The question whether these embryos were actually complete will be considered when we come to deal with the various larval organs.

Both Driesch and Crampton make the claim that single blastomeres of the 4-cell stage of the ascidian egg may give rise to entire larvæ. This is a crucial test of their views, for while it is possible and I believe practically certain that all their "complete larvæ of half size" were derived from the right or left halves of the egg and so included portions of all the various oöplasmic substances, this explanation could not apply to their quarter embryos. Driesch figures a larva with all the principal organs (his Fig. 16), which he says is derived from one of the first four blastomeres. However, in size it is as large as any of the half larvæ which he figures, and I have no doubt that it is such.

Crampton figures correctly the early cleavages of one of the anterior quadrants and he gives two figures of quarter larvæ, probably of an advanced stage; these figures, however, show no structure whatever save that there is an outer layer around the embryo. There is absolutely no evidence that these embryos are complete. Crampton calls attention to the fact that the long axes of these quarter embryos "are approximately parallel to the principal dorso-ventral axis of the original egg," a fact which I also can confirm. (See my Figs. 66, 69, 70.) He does not, however, determine the fact, which he apparently assumes, that the long axes of these quarter embryos correspond to the long axis of a normal embryo. This is actually not true, as I have shown; the long axes of the quarter embryos are not antero-posterior in direction but dorso-ventral and there has not therefore been any shifting of the axes nor of the oöplasmic substances of these quarter embryos.

Whether a larva derived from the right or left half of the egg is complete or not can be determined only by a study of the various systems of larval organs. It is evident that parts of all organs which are normally formed along the median plane (first cleavage plane) would appear in an embryo derived from one of the first two cleavage cells, even if the development were strictly partial; the really decisive test as to whether such an embryo is complete

or not must be found in the study of those organs which do not lie along the median plane.

a. Neural Plate and Sense Organs.

Chabry says that he never saw a partial embryo in which the neural plate had invaginated; on the contrary the nervous system always remains spread out in the form of a layer or plate; this plate occupies the face of the embryo which is morphologically median in position (its normal location), while the sense spots consist of pigmented cells which are superficial in position and which lie near the base of the tail. This agrees very closely with my observations, though I have frequently seen the neural plate invaginate by an irregular process. The eye is said by Chabry to be formed on the right side normally, but the fact that it may appear in the left half embryo leads him to conclude that its rudiment exists in the left half of the egg also. He thinks that the otolith comes only from the right posterior cell. I have not determined the exact cell origin of the sense organs in the normal larva, but in the partial larvæ they are formed only from the anterior quadrants and from either the right or left sides. I have not been able to distinguish between the eye and the otolith in the partial embryos of Cynthia.

Driesch says nothing of the neural plate nor of the manner in which the nervous system is formed in his small larvæ, though he mentions the fact that "the sense vesicle with the eye and otolith are not formed in the typically clear manner characteristic of the normal development." He found the eye spot almost always present, the otolith very seldom and he concludes that it makes no difference in the presence or absence of the sense organs whether the embryo has developed from certain cells of the 4-cell stage rather than from others. Since Driesch expressly states that he never raised a quarter embryo beyond the stage of his Fig. 16, at which stage the sense organs have not appeared, and since neither his figures nor descriptions give any evidence that he has distinguished anterior or posterior quadrants from right or left ones, it would be interesting to know how he could determine that sense organs might be formed from any quadrant of the egg—a result entirely contrary to my observations.

b. Notochord.

Chabry supposed that the notochord arose from both the anterior and posterior quadrants of the egg. Castle ('96) held that a single pair of cells of the posterior quadrants, $B^{8.6}$, "the posterior chorda fundament," were the only cells of the posterior quadrants which entered into the formation of the notochord. I am of the opinion that this cell is a mesenchyme and not a chorda cell (see Conklin, '05[1]), but even if it should be found to be a chorda cell it is only one cell of nine on each side of the mid line which give rise to that structure, while eight pairs of chorda cells come from the anterior quadrants. Certain it is that no trace of a notochord ever arises from the posterior cells when they are isolated, whereas chorda cells always arise from isolated anterior cells, though a notochord is rarely formed in such cases. Chabry describes (p. 294, Fig. 118) an anterior two-quarters embryo in which a naked chorda was seen in the perivitelline space outside the body of the embryo; such a case somewhat resembles the one shown in my Fig. 72. However, in every other instance which I have observed the chorda cells of an anterior embryo do not give rise to a notochord, but after escaping from the body of the embryo lie free in the perivitelline space as scattered cells. (Figs. 52, 66–70.)

But while a notochord is rarely or perhaps never formed in an anterior embryo and never in a posterior one, it is invariably found in a right or left one, and the figures of Chabry and Driesch as well as my own show that the process of formation is essentially the same as in a normal embryo. Chabry indeed believed that the notochord was primitively double and that half of it arose from each lateral half of the egg. He speaks of the fact that in Ascidia and Botryllus it is composed of a double row of cells and Crampton also refers to the fact that in the normal ascidian tadpole there are two rows of chorda cells, whereas Driesch has well said that in its fully formed condition the ascidian notochord is normally composed of a single row of cells. I find, as did Driesch, that the notochord of a lateral embryo is formed by interdigitation, just as in the normal embryo, but I also find, as opposed to Driesch that the notochord is never formed from any cells save the chorda cells which come from the posterior part of the gray crescent. Furthermore, my observations show, as did Chabry's, that the

formation of a tail is dependent upon the development of a notochord.

c. Muscles and Mesenchyme.

Chabry paid no particular attention to the number and location of the muscle cells in his partial larvæ, though he frequently speaks of their presence as being proved by the twitchings of the tail; these movements are less energetic than in normal larvæ and, as a consequence, partial larvæ do not escape from the egg membranes. Driesch also found that partial larvæ rarely hatch, probably because of their weak muscular movements, but he, too, paid no attention to the number and position of the muscle cells. Owing to the brilliant color of these cells in Cynthia they are recognizable at all stages; in the partial larvæ they are found only along one side of the notochord, where they form the characteristic three rows of cells, whereas the muscle cells of the opposite side are entirely lacking. In the oldest larvæ a few of the muscle cells extend around the end of the notochord to the side on which they were lacking. I have not been able to determine whether the number of muscle cells is actually increased during this process or merely rearranged, but I believe that the whole process consists in the moving of certain cells over to the side on which they were lacking, without any increase in their number. This is part of that process of regulation which begins with the rounding up of the surviving blastomere after the other one has been killed. In fact, this very extension of the muscle cells around the end of the notochord begins in this rounding up of the surviving blastomere and in that slight change in the direction of division which causes the median cells of the yellow crescent to lie nearer the middle of the first cleavage plane than in the normal egg. (Fig. 15.)

Chabry found (p. 308, Fig. 132) only one atrial invagination and one organ of fixation (papilla) in right or left half embryos. Driesch did not determine the number of atrial invaginations but he does call attention to the fact that but one papilla is present in embryos from isolated blastomeres. I have not observed the formation of the atrial invaginations or of the papillæ in Cynthia; even in the normal larvæ they are inconspicuous at the time of the metamorphosis and I have not studied them before that period. However, the areas of trunk mesenchyme in which the atrial invaginations appear, are conspicuous areas of clear, slightly

yellow, protoplasm in front of the muscle rows on each side of the tail; these areas may be recognized in the early cleavage stages and in no case are both these mesenchyme areas present in right or left half embryos. It is almost certain, therefore, that only one atrial invagination is formed in such embryos.

We find, therefore, that those parts of the larva which normally lie on the right side are missing in a left half embryo and those which normally lie on the left side are not found in a right half embryo, whereas unpaired organs which lie along the median plane are represented in both lateral half embryos. This is exactly what might be expected from a study of the organization of the egg since the substances, which give rise to median organs, are found along the median plane in both right and left blastomeres, whereas the materials which give rise to organs of the right side are found only in the right blastomere, those which give rise to organs of the left side, in corresponding positions in the left blastomere.

Neither Driesch nor Crampton attempt to show that a larva from the right half of an egg has the organs of the left side and this is the whole question at issue; if it does have these organs it is a complete embryo; if it lacks them it is a partial embryo, even if it does have a head and a tail. Chabry found that a larva from one of the first two blastomeres had a head and tail and median organs, but that it did not have the organs of the missing side and this conclusion I can entirely confirm.

All of the muscle substance (myoplasm) and most of the mesenchyme (chymoplasm) is localized in the posterior half of the egg, and corresponding to this distribution we find that an anterior half embryo entirely lacks muscles, though it may have a small amount of mesenchyme (that derived from the cell $A^{7.6}$), whereas a posterior half embryo contains a large number (probably the full normal number) of muscle cells and most of the mesenchyme.

V. REGULATION IN THE ASCIDIAN EGG AND EMBRYO.

It is well known from the work of Loeb ('92) and L. Schultze ('99) that the brain of Ciona will be regenerated when extirpated in the adult animal, and that the siphons will be restored when they are cut off. Driesch ('02) has also shown that Clavellina has extraordinary powers of regenerating almost all lost parts.

This power of regeneration in the adult is in striking contrast with its lack in the egg and embryo and requires some explanation.

It should not be overlooked that such injuries to the egg and embryo as have been described in the preceding pages are probably more extensive and far-reaching than any which are capable of being repaired in the adult. As Chabry says the destruction of one of the first two blastomeres is the same in its effect as the destruction of the right or left half of the body of an adult. The destruction of the anterior half of the egg is similar to the total loss of the nervous system and notochord of the larva; while the death of the posterior half corresponds to the destruction of the whole of the muscular system and most of the mesenchyme of the larva, since in each case the specific substance which alone gives rise to these organs is destroyed. Therefore these injuries are probably much more extensive than any which have been practiced on the adult animal.

Furthermore, I am of the opinion that the extremely rapid development of the ascidian egg and embryo may itself act as a check on regulation. In Cynthia and Ciona the fully formed larval stage is reached in about twelve hours after the fertilization of the egg, and these larvæ usually undergo metamorphosis into the adult form within the next twelve hours. In Molgula the development is even more rapid. It seems to me probable that the restoration of the parts of the missing right or left half of a larva might be fully accomplished if the larval life were longer. In a right or left half larva one day old the ectoderm cells have closed over the injured side, the notochord is complete, the neural plate has invaginated, although abnormally, and the muscle cells have begun to grow over from the uninjured to the injured side. There is here evidence of considerable regulative ability and it seems to me possible that, with more time before the metamorphosis, complete rows of muscle cells might be found on both sides of the tail and that the mesenchmye cells might grow over to the side on which they are lacking and an atrial invagination appear in them.

Inasmuch as the only form of regulation shown by the ascidian egg or embryo is this overgrowth of cells from the uninjured to the injured side, it is probable that no amount of time would ever suffice to produce an entire larva from the anterior or posterior half of an egg or from a quarter or any smaller portion. As a

matter of fact there is not the slightest indication in an anterior half embryo of any attempt to restore the missing myoplasm or muscle cells, nor does a posterior half embryo show any tendency to form chorda-neuroplasm or neural plate or chorda cells. So far as observation and experiment show, each oöplasmic substance is capable of giving rise only to one particular kind of organ or tissue.

The question may be raised whether the presence of the injured blastomere within the chorion may not influence the development of the surviving cells and possibly prevent regeneration. In this and in all previous experimental work on the ascidian egg these injured cells have been left within the chorion in contact with the surviving cells and in this respect all work on these eggs has been done under similar conditions. Owing to the presence of the chorion it is practically impossible to remove the injured cells, and I am therefore unable to furnish an experimental test of the influence or lack of influence of these cells upon the surviving ones. However, there is sufficient evidence, I think, to show that it is not the presence of these cells which prevents regeneration. Contact with the injured cell might be expected to hinder or prevent the closing of the surviving half along the injured side, but it is just this form of regulation, and this only, which is manifested by these eggs. The presence of the injured cells can have nothing to do with the failure of the anterior half embryo to form a tail, or the posterior half embryo, a head; on the other hand, I have shown conclusively that the development of a tail is dependent upon the presence of a notochord, and the formation of a head upon the presence of the gastral endoderm and neural plate. The only possible influence of the injured cell upon the surviving one would be to limit the form-regulation; but as I have said this it does not do. It is inconceivable that the presence of the injured cell should prevent the myoplasm from giving rise to other organs than muscles, or the chorda-neuroplasm to other organs than chorda and neural plate.

These injured cells are rarely killed, but they remain transparent and entire, although quiescent; they do not decay and form a nidus for bacteria and I am convinced that their presence does not materially influence the development of the surviving half nor limit its powers of regulation.

VI. GENERAL CONCLUSIONS.

The conclusions which follow from these experiments are so obvious that they need but little emphasis here. Not only is the fact established that individual blastomeres give rise only to those parts of an embryo which they would produce under normal conditions, but the cause of this is clearly indicated. The development of the ascidian egg is a mosaic work because individual blastomeres are composed of different kinds of oöplasmic material; this mosaic work is not merely a cleavage mosaic but also a mosaic of germinal substances, several of which are recognizable before cleavage begins.

1. *Organ-Forming Substances.*

I have elsewhere shown that at least five distinct kinds of oöplasm are recognizable in the egg of Cynthia before the first cleavage and that all of these substances are localized in their final positions as early as the close of that cleavage. In these experiments I have not been able to isolate the different oöplasmic substances in the unsegmented egg, but after the second or third cleavages several of these substances may be isolated and in such cases each substance gives rise only to a definite kind of tissue or organ, and apparently it has no power to produce any other kind. The myoplasm produces muscle cells only; the chorda-neuroplasm, only chorda and neural plate cells; the chymoplasm, only mesenchyme; the endoplasm and ectoplasm only endoderm and ectoderm, respectively. Whenever an isolated blastomere lacks any of these substances, the embryo which develops from that blastomere lacks the corresponding organs. Accordingly the potencies of individual blastomeres are dependent upon the oöplasmic substances which they contain; the prospective value of any blastomere is not primarily a function of its position, but rather of its material substance.

The reason that the anterior quadrants of the egg never produce muscle cells is evidently due to the fact that they totally lack the yellow myoplasm; the fact that the posterior quadrants never produce a neural plate or chorda, is evidently due to the complete absence of the chorda-neuroplasm in these quadrants; the cells of the ventral (animal) pole produce only ectoderm, without a trace of endoderm or mesoderm,—evidently because these cells are composed almost entirely of clear ectoplasm.

Experiment confirms, therefore, what observation of the normal development plainly indicates that these strikingly different oöplasmic substances are not totipotent, but that as early as the close of the first cleavage and probably much earlier, they are differentiated for particular ends, and that if they develop at all they give rise to organs of a particular kind. These materials are, therefore, "organ-forming substances" and the areas of the egg in which they are localized are "organ-forming regions."

I need not here point out the similarity between this conclusion and the well-known theories of Sachs and His, nor the differences between my results and the commonly accepted view that the egg is composed of "simple undifferentiated protoplasm" or that "cleavage is a mere sundering of homogeneous materials capable of any fate," or that "the prospective value of a blastomere is a function of its position." Whatever may be true of other animals these things are certainly not true of ascidians.

While there are few, if any, other cases known in which the differentiations of the oöplasm are so striking or so numerous as in the egg of Cynthia there can be no doubt that organ-forming substances are present in the eggs of many animals. In particular the works of Fischel ('97, '98, '03) on the Ctenophore, of Boveri ('01) on Strongylocentrotus; of Wilson ('04) on Dentalium and Patella and of Conklin ('03) on Physa, Planorbis and Limnæa have shown that distinct kinds of protoplasm are present in these eggs which are destined in the course of development to give rise to particular germ layers or organs. In the light of these discoveries it can scarcely be doubted that the general cause of mosaic development is to be found in the presence in the egg or blastomeres of distinct kinds of protoplasm, or of organ-forming substances.

2. *Localization of Oöplasmic Substances.*

The three principal substances in the egg of Cynthia, viz: the clear, the yellow and the gray, are already present and localized in the oöcyte before it escapes from the ovary. The yellow (mesoplasm) forms a peripheral layer around the entire egg; the clear (ectoplasm) is the clear achromatic substance within the germinal vesicle; the gray (endoplasm) constitutes most of the

remainder of the egg.[1] For the sake of brevity this earliest form of localization may be described as concentric or spherical, although the germinal vesicle does not lie exactly in the center of the egg but is slightly eccentric toward one pole.

During maturation and fertilization this concentric localization gives place to a polar or radial form. Immediately after the entrance of the spermatozoon into the egg the peripheral layer of yellow mesoplasm flows rapidly to the lower pole where it collects in the form of a cap; the clear ectoplasm which escapes from the germinal vesicle at first lies at the animal pole where it surrounds the maturation spindles but after the entrance of the spermatozoon it also flows to the lower pole where it collects into a layer or stratum just above the mesoplasm; the gray endoplasm after these movements occupies almost all of the upper half of the egg. The egg at this stage appears to be radially symmetrical, the three principal substances being arranged in strata at right angles to the egg axis.

Soon after the entrance of the spermatozoon this radial form of localization gives place to a bilateral one; the sperm nucleus and aster move up to the equator of the egg along one meridian which further development shows to be the median plane on the posterior side; the clear and yellow substances also move to the posterior pole along with the sperm nucleus and the yellow substance here forms a crescent around the posterior side of the egg, just below the equator. At this stage the egg is bilaterally symmetrical, there being but one plane which will divide equally all of the oöplasmic substances.

Finally during the first cleavage this early bilateral localization is changed into the definitive localization which is characteristic of all stages up to the late gastrula. The yellow crescent remains in the position which it occupied before the first cleavage and here gives rise to muscle and mesenchyme cells; the clear protoplasm comes to occupy most of the ventral hemisphere and gives rise to ectoderm; the gray substance occupies the dorsal hemisphere in

[1]Although I have not been able to isolate these various oöplasmic substances before cleavage begins and, therefore, can bring no experimental evidence to prove that they are organ-forming substances at this early stage, it nevertheless seems probable that materials which are identical in color and texture with the organ-forming substances of later stages, to which they directly give rise, are also similar in potency. There is no apparent reason for believing that these strikingly different kinds of oöplasm of the ovarian egg are any less distinct or more nearly totipotent than during the cleavage stages.

front of the yellow crescent and its anterior portion becomes the gray crescent of chorda-neuroplasm, while its posterior portion is the deep gray endoplasm which gives rise to the gastral endoderm.

The form of localization of these substances, therefore, undergoes marked changes during the fertilization and first cleavage; it is concentric in the oöcyte, polar or radial immediately after the entrance of the sperm, bilateral just before the first cleavage, and definitive at the close of the first cleavage.

I have elsewhere ('05[1]) shown reason for believing that even in the stage of radial localization in the egg of Cynthia there is probably some structural peculiarity of the egg which determines that the path of the sperm shall lie in one meridian rather than in another and therefore that the median plane of the embryo and its posterior pole are not determined by the chance movements of the sperm within the egg. Similarly the basis for polar or radial localization is present in the ovarian egg in the slight eccentricity of the germinal vesicle toward the animal pole, though the oöplasmic substances are largely localized in concentric form at this stage. I am unable to determine whether any structural basis for bilateral localization exists in the ovarian eggs of ascidians, but inasmuch as the localization invariably becomes bilateral at a later stage it seems necessary to suppose that there is some such intrinsic determinative factor.

In almost every group of animals the chief axis of the egg is already marked out in the oöcyte, the pole toward which the germinal vesicle is eccentric becoming later the animal pole of the egg and the ectodermal pole of the embryo. Despite this eccentricity of the germinal vesicle the localization of oöplasmic substances in the oöcyte of ctenophores, nemertines, echinoderms and ascidians is chiefly concentric, the polar localization of these substances first appearing during the maturation and fertilization. On the other hand Wilson ('04) has found a markedly polar localization of the oöplasm in the oöcyte of Dentalium; while it is probable that in the oöcytes of insects and cephalopods the localization is bilateral in form.

Boveri ('01) found that distortion of the egg of Strongylocentrotus after the formation of the equatorial zone produced no change in the polar stratification of the egg nor in the potencies of its different substances. Wilson ('03), Yatsu ('04) and Zeleny ('04) have discovered that fragments from any part of the egg of

Cerebratulus before maturation may give rise to entire larvæ; whereas this is not usually the case after maturation and fertilization, the potencies of the substances at the animal and vegetal poles being different. It is evident that during the concentric stage of localization section of an egg in any plane would leave samples of all the oöplasmic substances in each piece; in the stage of polar-radial localization any section of the egg parallel with the egg axis would leave samples of all the oöplasmic substances in each piece; in the bilateral stage, only the right or left halves would contain parts of all the substances. Probably one important reason why parts of eggs may give rise to whole embryos in some cases and not in others may be found in the fact that at the time of the experiment the form of localization may have been concentric in some cases, radial in others and bilateral in still others. (See Boveri, '01; Wilson, '04[1].)

3. *Cleavage and Localization.*

In previous publications ('05[1], '05[2]) I have pointed out the fact that localization precedes cleavage in the ascidian egg and that the localization pattern does not closely coincide with the cleavage pattern. On the other hand there is normally a constant relation between particular cleavage planes and the various oöplasmic substances. The first cleavage always lies in the median plane and bisects all the oöplasmic substances; the second is transverse to the median plane and separates the yellow crescent from the gray one; the third cleavage is at right angles to the two preceding ones and separates the clear ectoplasm of the ventral hemisphere from the different substances of the dorsal hemisphere. Probably in no other animal is the cleavage so constant and so perfectly bilateral as in the ascidians and yet even here the position and direction of the cleavage planes is less constant than the form of localization.

Among annelids and mollusks, as is well known, the cleavage is typically spiral and in many cases it is radially symmetrical. This radial symmetry of cleavage does not indicate, however, that the localization of oöplasmic substances is also radially symmetrical, for in some cases this localization is known to be bilateral and this is probably true in all cases. (See Conklin, '05[1], pp. 90–92.)

The relation of the cleavage planes to this bilateral organization

is very different in cases of spiral and of bilateral cleavage, and consequently the results of killing any one or more of the first four blastomeres may vary in different cases; in general there is less likelihood of obtaining an entire embryo from an isolated blastomere of spiral cleavage than from one of the first two blastomeres in bilateral cleavage.

In other cases the cleavage planes bear no constant relation to the planes of localization. Thus in the frog's egg the first cleavage may lie in the median plane or at varying angles to this plane and Brachet ('04) has recently shown that the character of an embryo derived from one of the first two blastomeres depends entirely upon the relation between the first cleavage plane and the median plane of organization.

It is probable that the bilaterality of organization is no more perfect in ascidians than in annelids, mollusks or amphibians, but the bilaterality of cleavage is much more perfect. Accordingly, each of the first two blastomeres of the ascidian egg always contains half of every oöplasmic substance, in the frog's egg it may or may not contain half of these substances, in the annelid or mollusk it never does.

I agree therefore with Brachet ('04) and Wilson ('04[1], '04[2]) that the varying results of experiments on the potencies of blastomeres are due in part to the varying relations of cleavage to localization, and in part also to the different types of localization (concentric, radial, bilateral) in different eggs.

4. *Determinate and Indeterminate Cleavage and Development.*

In a great many animals belonging to phyla as widely separate as Ctenophora, Polyclada, Nemertinea, Nematoda, Rotifera, Annelida, Mollusca, Arthropoda and Tunicata the cleavage of the egg is constant in form and differential in character and under normal conditions, definite cleavage cells always give rise to definite structures of the embryo or larva. For this type of cleavage I proposed several years ago ('97, '98) the designation "determinate." In a few animals the cleavage is known to be extremely irregular, as in Pennaria (Hargitt, '04), Renilla (Wilson, '84), and probably also in planarians (Hallez, '87; Stevens, '04), while in other cases it is unknown whether the cleavage is normally constant and differential or not (Echinoderms); in still other cases

the planes of cleavage bear no constant relation to the planes of localization, as in the eggs of some of the vertebrates (frog, fish). For all such cleavages I proposed the name "indeterminate," but at the same time I was careful to state that this was "to be understood as applying only to the cleavage, for in its main features and results the development of all animals is determinate; that is, predictable. Even in cnidaria, echinoderms and vertebrates there appears successively a blastula, gastrula, larva, and adult of determinate form and character" ('98, p. 21).

But while the cleavage is indeterminate in some cases there is reason to suppose that there is a definite organization of the egg in all animals—in short that the organization of the individual is determinate at all stages from the egg to the adult. Even in such an egg as that of Pennaria it is certain that there must be determinative factors somewhere, if not in the cytoplasm then in the nucleus, which determine that the egg shall develop into a Pennaria rather than into some other animal; and it is further evident that these determinative factors must be present in the cytoplasm at a relatively early stage, if not at the very beginning of development.

In the echinoderm egg, which was at one time supposed to be homogeneous or isotropic, Boveri ('01) has shown that a polar-radial localization of at least three distinct morphogenetic substances takes place immediately after maturation, and in this case, as in the ascidians it is probable that there is an earlier concentric localization of these substances in the oöcyte. Since these three substances are localized in zones or strata, one above the other, around the chief axis of the egg, they are all present in each of the first four blastomeres of the egg, each of which may give rise to an entire embryo; but when they are isolated each is found to be strictly limited in its potentialities.

While therefore there are several groups of animals in which the cleavage is indeterminate there are few or none in which the oöplasm is isotropic; on the contrary in almost every phylum the eggs and blastomeres show differentiations and localizations of the oöplasm which are of morphogenetic value. "Everywhere," as Fischel ('03) has well said, "the fundamental principle of normal development is a mosaic work." But while Fischel supposes that "only the materials for the primitive organs of the embryo are preformed in the egg cell and that the material substratum for the differentiation of the special organs is probably first formed during

the later stages," I find that in the ascidian egg all the principal organs of the larva are represented by distinct organ-forming substances which are localized in their definitive positions and proportions as early as the close of the first cleavage.

There is a world-wide difference between such results as these and those which were reached by Driesch and some of the earlier workers in this field. Wilson ('04) has recently expressed the opinion that "had the experimental analysis of cleavage been first undertaken in the case of such a determinate type as that of the gasteropod or annelid and had Roux not handicapped his theory with a purely speculative hypothesis of differentiation, which proved to be untenable, the whole discussion would have taken a different course; and I believe it would from the first have been recognized that the mosaic principle holds true in greater or less degree for every type of development, not excepting the most 'indeterminate' forms of cleavage." Considering the fact that such highly determinate forms as the ascidian and the ctenophore were studied in some of the earliest experiments on the potency of cleavage cells, I am of the opinion that the course which this discussion took was not primarily due to the fact that work began on relatively indeterminate forms. On the other hand I am convinced that the whole trend of opinion on the organization of the egg and on the potency of cleavage cells would have been different if those who did this work had been more familiar with the normal development of the forms studied, and in their zeal for the experimental method had not discarded the old and approved method of observation. It has taken such careful observers of normal processes as Boveri and Wilson to apply most successfully the experimental method to the problem of the organization of the egg, and the results of such work constitute a well-deserved tribute to the permanent value of the work of Roux.

SUMMARY.

I. *Normal Development.*

1. In the ovarian egg of Cynthia (Styela) partita there are three strikingly different kinds of oöplasm, viz: a superficial yellow layer, a central gray area, and a large transparent germinal vesicle. At this stage the localization of these substances is approximately concentric.

2. During maturation and fertilization the yellow substance flows rapidly to the vegetal pole where it forms a superficial layer or cap; the clear substance escapes from the germinal vesicle and flows toward the vegetal pole where it forms a stratum above the yellow cap; the gray substance occupies the animal half of the egg. The localization at this stage is polar-radial.

3. The sperm nucleus which lies in the center of the yellow cap moves to the posterior pole of the egg and the yellow and clear substances move with it. The yellow material here forms a crescent which lies with its center at the posterior pole and its arms extending forward on each side about halfway around the egg; the clear substance forms a band just above and internal to the crescent; the gray substance occupies the remainder of the egg. At this stage the localization is bilateral.

4. The first cleavage furrow appears in the plane of bilateral symmetry and divides each of the oöplasmic substances equally. At the close of this cleavage the clear substance occupies the animal (ventral) half of the egg; the gray substance lies at the middle of the vegetal (dorsal) pole while around the posterior border of the dorsal hemisphere is the yellow crescent and around its anterior border is a light gray crescent. This is the definitive localization of these substances, and in these positions the clear material gives rise to ectoderm, the gray to endoderm, the yellow crescent to muscles and mesenchyme, and the gray crescent to chorda and neural plate.

5. The second cleavage is transverse to the antero-posterior axis and separates the gray crescent from the yellow; the third cleavage separates the clear protoplasm of the ventral hemisphere from the various substances of the dorsal hemisphere.

II. *Experiments.*

6. Individual blastomeres were injured by spurting or shaking the eggs in the 2, 4, 8, or 16-cell stages. The surviving blastomeres were then studied both in the living condition and after being stained and mounted.

7. *Cleavage.* Isolated blastomeres always segment as if they still formed part of the whole, except that the direction of some of the cleavages is slightly altered so that the resulting cell mass is more nearly spherical than in the normal egg. These alterations

in the direction of cleavage and the consequent closing of the injured side are more apparent in isolated blastomeres of the 2-cell stage than in those of later stages.

8. *Gastrulation.* In right or left or anterior halves, gastrulation usually proceeds as if the fragment still formed part of the whole; even though the gastrula may be rounded in form the location of the different substances shows that it is strictly partial. Not infrequently isolated blastomeres give rise to exogastrulæ, which ultimately right themselves. In posterior halves and in quarter embryos, gastrulation does not at all resemble the normal process, either in methods or results.

9. *Right or Left Half Embryos.* A lateral half embryo is usually closed along the injured side; it has a head and a tail; a typical notochord, which is formed only from the chorda cells of the surviving side, and which is therefore composed of half the normal number of cells; an atypical neural plate and sense vesicle, formed only from the typical neural plate cells of the surviving side; a typical mesenchyme area in which the atrial invagination of one side is formed and three typical rows of muscle cells on one side of the notochord, but none along the injured side. In the latest stages to which these lateral embryos were reared (corresponding to the period of metamorphosis in normal larvæ) the muscle cells have begun to grow around the hinder end of the notochord to the side on which they were lacking; but in no case are the three rows of the normal embryo present on this side. Probably only one atrial invagination and one papilla are ever formed in these lateral embryos. These are therefore half embryos in which some cells have grown over from the uninjured to the injured side, but in which absolutely no change has taken place in the potency of the individual cells or of the different oöplasmic substances.

10. *Anterior Half Embryos.* Embryos derived from the two anterior quadrants of the egg have no trace of muscle cells nor of muscle substance; although the normal number of chorda cells are present they rarely if ever form a notochord but usually escape from the body of the embryo and lie scattered in the perivitelline space; the neural plate cells are present in normal number and position but the plate rarely, if ever, invaginates to form a sense vesicle; in late stages sense spots are formed from certain cells of the neural plate; cells of the gastral endoderm and general ectoderm are frequently present in their normal positions and numbers. A tail is never formed in these anterior embryos and they bear no resemblance whatever to a normal larva.

11. *Posterior Half Embryos.* Embryos derived from the two posterior quadrants have no trace of notochord or of chorda cells, neural plate, sense vesicle, sense spots, or gastral endoderm; they contain a mass of muscle and mesenchyme cells and a double row of caudal endoderm cells, as in the normal embryo. There is no indication of a tail or head, the embryo remaining rounded in form as long as it lives.

12. *Three-Quarter Embryos.* Embryos derived from three of the first four blastomeres are more nearly perfect than are half embryos, but they always show defects corresponding to the missing blastomere. If an anterior blastomere is killed, the neural plate and sense vesicle of the resulting larva are atypical and the notochord lacks the normal number of cells; if a posterior cell is killed, the muscle and mesenchyme cells are lacking along one side of the tail.

13. *One-Quarter Embryos; Two-Quarter Diagonal Embryos.* Embryos derived from any one quadrant or from two diagonal quadrants of the egg are always very defective. They never have a notochord, though if they come from anterior quadrants they may give rise to scattered chorda cells in the perivitelline space; there is never a sense vesicle, though if they are from an anterior quadrant a neural plate and sense spots are present. The posterior quadrants always contain muscle, mesenchyme and caudal endoderm cells, but never a trace of notochord, neural plate nor sense spots. The embryos are always rounded, there being no distinction of head and tail, and in no respect do they resemble normal larvæ.

14. *Eighth and Sixteenth Embryos.* When blastomeres are injured in the 8-cell or 16-cell stages a great variety of abnormal forms are produced. Ventral blastomeres give rise only to rounded masses of ectoderm cells in which there is no trace of endoderm or mesoderm; posterior dorsal cells give rise only to muscle, mesenchyme, and caudal endoderm; anterior dorsal cells to neural plate, chorda, and gastral endoderm.

15. *Anterior and Posterior Half Gastrulæ.* When cup-shaped gastrulæ of the stage shown in Fig. 8 are cut in two transversely so as to leave all of the yellow cells in one half and all of the chorda

and neural plate cells in the other a notochord, sense vesicle, or tail is never formed and nothing resembling a normal larva develops from either half. The anterior half never contains muscle cells; the posterior half contains many muscle and mesenchyme cells, but evidently no chorda or neural plate cells.

III. *Conclusions.*

16. My results confirm and extend those of Chabry, but they are fundamentally unlike those of Driesch; I agree with the work of Crampton as to the cleavage of isolated blastomeres but cannot agree with him that whole embryos or larvæ are ever formed from isolated blastomeres of the ascidian egg.

17. Regulation in the ascidian egg and embryo is limited to the closing of the embryo and the consequent extension of certain cells from the uninjured to the injured side; and also to the formation of a typical notochord and an atypical sense vesicle in right or left half embryos. One oöplasmic substance never gives rise to another nor does a given type of cell ever produce cells of another type or organs of a different kind than those which would arise from it in a normal embryo. The fact that the power of regulation is apparently greater in the adult ascidian than in the egg or embryo may be deceptive; the injury to the egg which wipes out completely certain oöplasmic substances may be really greater than any which may be repaired in the adult. Furthermore it is possible that the very rapid development of ascidians may act as a check on regulation.

18. These results prove that at least five of the substances which are present in the egg at the close of the first cleavage, viz: ectoplasm, endoplasm, myoplasm, chymoplasm, and chorda-neuroplasm, are organ-forming substances. They develop, if they develop at all, into the organs which they would normally produce; and conversely, embryos which lack these substances, lack also the organs which would form from them. Although I have been unable to test the potencies of these substances before cleavage begins, there seems to be no reason for supposing that they are ever totipotent. Three of these substances are clearly distinguishable in the ovarian egg and I do not doubt that even at this stage they are differentiated for particular ends.

19. A possible explanation of the fact that all the fragments of an immature egg may give rise to entire larvæ, whereas the proportion which gives rise to larvæ steadily decreases after maturation and fertilization, may be found in the fact that before maturation the localization of oöplasmic substances is usually concentric, after maturation and fertilization, polar-radial; while just before or shortly after the first cleavage the localization may become bilateral. Also the fact that isolated blastomeres may give rise to whole embryos in some animals and to partial ones in others may be due to the varying relations of cleavage planes to localization planes. If at the close of the second cleavage the localization is still radially symmetrical, each of the first four blastomeres would probably be capable of giving rise to an entire larva; if the first cleavage invariably lies in the plane of bilateral symmetry, as in ascidians, each of the first two blastomeres might be capable of giving rise to an entire larva (though my work shows that this would not necessarily happen); if the cleavage planes do not coincide with the planes of localization, as in mollusks and annelids, isolated blastomeres would not give rise to entire larvæ. (See Wilson, '04[1], '04[2].)

20. The development of ascidians is a mosaic work because there are definitely localized organ-forming substances in the egg; in fact the mosaic is one of organ-forming substances rather than of cleavage cells. The study of ctenophores, nemertines, annelids, mollusks, ascidians and amphibians (the frog) shows that the same is probably true of all these forms and it suggests that the mosaic principle may apply to all animals. (*cf.* Fischel, Wilson.)

LITERATURE CITED.

Barfurth, D., '93.—Halbbildung oder Ganzbildung von halber Grosse? Anat. Anz., viii.

Beneden, van et Julin, '84.—La segmentation chez les ascidiens dans ses rapports avec l'organization de la larve. Arch. de Biol., v.

Boveri, Th., '01.—Ueber die Polaritat des Seeigeleies. Verh. Phys. Med. Ges., Würzburg, xxxiv.

'01.—Die Polaritat von Ovocyte, Ei und Larve des Strongylocentrotus lividus. Zool. Jahrb., xiv.

Brachet, A., '04.—Recherches expérimentales sur l'oeuf de Rana fusca. Arch. de Biol., xxi.

CASTLE, W. E., '96.—The Early Embryology of Ciona intestinalis, Flemming L. Bull. Mus. Comp. Zool., xxvii.

CHABRY, L., '87.—Contribution à l'embryologie normale et teratologique des Ascidies simples. Journ. Anat. et Physiol., xxiii.

CONKLIN, E. G., '97.—The Embryology of Crepidula. Jour. Morph., xiii.

'98.—Cleavage and Differentiation. Woods Hole Biol. Lect.

'03.—The Earliest Differentiations of the Egg. Science, xvii.

'05[1].—The Organization and Cell-Lineage of the Ascidian Egg. Jour. Acad: Nat. Sci., Philad., xiii.

'05[2].—Organ-Forming Substances in the Eggs of Ascidians. Biol. Bull., viii.

CRAMPTON, H. E., '97.—The Ascidian Half-Embryo. Ann. New York Aca. Sci., x.

DRIESCH, H., '95.—Von der Entwicklung einzelner Ascidienblastomeren. Arch. Entw. Mech., i.

'02.—Studien über das Regulationsvermögen der Organismen. 6 Die Restitutionen der Clavellina lepadiformis. Arch. Entw. Mech., xiv.

'03.—Ueber Anderung der Regulationsfahigkeiten im Verlauf der Entwicklung bei Ascidien. Arch. Entw. Mech., xvii.

FISCHEL, A., '97, '98.—Experimentelle Untersuchungen am Ctenophorenei. I and II Arch. Entw. Mech., vi, vii.

'03.—Entwicklung und Organ-Differenzirung. Arch. Entw. Mech., xv.

HALLEZ, P., '87.—Embryogénie des Dendrocoeles d'eau douce. Paris.

HARGITT, C. W., '04.—The Early Development of Pennaria tiarella. McCr. Arch. Entw. Mech., xviii.

HERTWIG, O., '92.—Urmund und Spina Bifida. Arch. Mik. Anat., xxxix.

'98.—Die Zelle, II. Jena.

KORSCHELT UND HEIDER, '02.—Lehrbuch der vergleichenden Entwicklungsgeschichte. Allgemeine Theil. Jena.

LOEB, J., '92.—Untersuchungen zur Physiologischen Morphologie der Thiere, vi and vii.

ROUX, W., '95.—Gesammelte Abhandlungen über Entwicklungsmechanik der Organismen. Bd. II, Leipzig.

'92.—Ueber das Entwicklungsmechanische Vermogen jeder der beiden ersten Furchungszellen des Eies. Verh. d. Anat. Ges. zu Wien.

'03.—Ueber die Ursachen der Bestimmung der Hauptrichtungen des Embryo im Froschei. Anat. Anz., xxiii.

SCHULTZE, L. S., '99.—Die Regeneration des Ganglions von Ciona intestinalis L. und über das Verhaltnis der Regeneration und Knospung zur Keimblatterlehre. Jena. Zeit., xxxiii.

STEVENS, N. M., '04.—On the Germ Cells and the Embryology of Planaria Simplicissima. Proc. Acad. Nat. Sci., Philad.

WEISMANN, A., '92.—Das Keimplasma. Jena.

WILSON, E. B., '84.—The Development of Renilla. Philos. Trans., clxxiv.

'03.—Experiments on Cleavage and Localization in the Nemertine Egg. Arch. Entw. Mech., xvi.

'04[1].—Experimental Studies on Germinal Localization, I and II. Jour. Exp. Zoöl., i.

'04[2].—Mosaic Development in the Annelid Egg. Science, xx.

YATSU, N., '04.—Experiments on the Development of Egg Fragments in Cerebratulus. Biol. Bull., vi.

ZELENY, C., '04.—Experiments on the Localization of Developmental Factors in the Nemertine Egg. Jour. Exp. Zoöl., i.

A FEW FACTS CONCERNING THE RELATIONSHIPS AND REPRODUCTION OF SOME BERING SEA TUNICATES.

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WHILE President Jordan was engaged, as commissioner in charge of the fur-seal investigations for 1896, in studying the natural history of the seals of the Pribilof Islands, he collected a considerable number of tunicates. These he kindly intrusted to me for study. They proved to be so interesting that during his second summer's work (1897) in the same capacity he encouraged the enthusiastic young zoölogists, R. E. Snodgrass, A. W. Greeley, and Trevor Kincaid, who accompanied him, to give particular attention to collecting these animals. The result was a large, well-preserved collection, the study of which contributes substantially, in several directions, to our knowledge of the group. These contributions will appear in detail as a part of the final report of the scientific investigations made by the commission, to be published later by the United States Government. Some of the facts brought to light are, however, of sufficient consequence to make worth while their publication in advance of the report itself. I consequently present them here. As indicated by the title of the note, they relate to the affinities of the Bering Sea tunicate fauna and to the reproduction of some of the species studied.

The collection contains eleven species, ten of which are new to science. These are distributed among seven genera in the following way: *Boltenia*, *Styela*, *Aplidiopsis*, and *Synoicum*, each one species; *Dendrodoa* and *Polyclinum*, each two species; and *Amaroucium*, three species. So far as I am able to determine, no tunicates have before now been described from this portion of the world, the northern species hitherto known having come from the North Atlantic and Arctic oceans, mostly