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ORGAN-FORMING SUBSTANCES IN THE EGGS OF ASCIDIANS.

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WITH 24 PHOTOMICROGRAPHS OF LIVING EGGS OF CYNTHIA (STYELA) PARTITA STIMPSON.

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That the egg of any animal is composed of "simple, undifferentiated protoplasm" is an article of traditional belief with a large number of zoölogists, and that the cleavage of the egg is "a mere sundering of homogenous materials capable of any fate" is a doctrine which has been given great prominence in recent years. In favor of these commonly accepted views stands a considerable body of experimental work on the development of the ovum; fragments of eggs or isolated blastomeres in many cases are said to give rise to entire larvæ, thus proving, as is usually claimed, that the parts of the egg or embryo are still undifferentiated at the time of the experiment.

But not all experiments on development have confirmed these conclusions; some of the first and most careful researches of this sort led to directly opposite results. In the development of the frog's egg Roux found (1883, 85, 87, 89, 92, 93, 94, etc.) that the median plane of the embryo is determined in the egg immediately after fertilization and that "the development is, from the second cleavage on, a mosaic work of at least four vertical independently developing pieces." In 1887 Chabry showed that the early cleavage cells of the ascidian egg are specified for particular ends and that they develop, if they develop at all, into parts which they would produce under normal conditions. These results were, however, denied on the ground of other ex-

perimental work, and by many it was held that the "mosaic theory had received its death blow from the facts of experimental embryology." Among the ova in which isolated blastomeres were found to be capable of complete development were those of ascidians and from this fact it was assumed that here also the early cleavage cells were undifferentiated.

Nevertheless, although some of the results of experimental embryology seemed to render the mosaic theory untenable, additionable evidence in favor of it was furnished by another line of work commonly known as "cell-lineage." In a considerable number of animals belonging to distinct phyla (annelids, mollusks, polyclades, nemerteans, nematodes, rotifers, crustaceans and ascidians) the cleavage of the egg was found to be constant in form and differential in character and each of the early cleavage cells was shown to play a perfectly definite part in the building of the embryo. By those who maintained the traditional view as to the simplicity of the egg and the homogeneity of the blastomeres this fact was explained as due to "the continuity of development," "the position of the blastomeres in the cell complex," etc. But this explanation was never a satisfactory one and is no longer tenable; both observation and experiment have shown conclusively that in certain eggs the blastomeres are not all alike. In particular the experimental work of Crampton (1896), Fischel (1897, 1898), Boveri (1901), Wilson (1903, 1904), Yatsu (1904) and Zelney (1904) has demonstrated that in ctenophores, echinoderms, nemerteans, mollusks and annelids all portions of the egg are not equipotential; this, as well as other work on the organization of the egg, proves that there is a differentiation and localization of the substances of the egg very unlike the "simple undifferentiated protoplasm" of traditional belief.

In most instances the protoplasm of the different blastomeres of an egg is much the same in appearance; in a few cases it is visibly different, but in all cases which have been carefully studied definite blastomeres always give rise to definite parts of the embryo. In fact the manner and rate of development as well as its results are so thoroughly characteristic of certain blastomeres that students of cell-lineage have usually concluded that the protoplasm of different blastomeres must differ, even though these differences

are not directly visible. Recent experimental work on some of these forms confirms and extends these conclusions and proves that even in the egg before cleavage begins different substances may be present which are destined in the course of development to enter into specific parts of the embryo.

The most notable differentiations of the oöplasm which have been observed hitherto are found in *Myzostoma* (Driesch, 1896; Wheeler, 1897; Carazzi, 1904), in *Strongylocentrotus* (Boveri, 1901), in *Unio* and *Chætopterus* (Lillie, 1901, 1902), in *Dentalium* and *Patella* (Wilson, 1904) and in the gasteropods *Crepidula*, *Physa*, *Planorbis* and *Limnæa* (Conklin, 1902, 1903). In none of these cases, however, are the differentiations and localizations of the oöplasm so remarkable as are those which occur in the ascidian egg. Here the different substances of the egg are strikingly dissimilar; they are localized in their definitive positions at a remarkably early period, and they may be traced with ease and certainty through the maturation and fertilization, the cleavage, the gastrulation and the later stages until they give rise to specific organs or parts of the larva.

Material and Methods.

I have studied the early differentiations of the egg in three species of simple ascidians, viz., *Cynthia* (*Styela*) *partita* Stimpson, *Ciona intestinalis* (L.) Flemming, *Molgula manhattensis* Verrill. The differentiations and localizations are essentially the same in all of these species, but as the different kinds of oöplasm are more brilliantly colored in *Cynthia* than in either of the other genera named, I shall devote particular attention to this form.

In an extensive publication on the organization and cell-lineage of the ascidian egg (Conklin, 1905), I have figured and described the remarkable localization of germinal materials in the egg of *Cynthia*. The differentiations of the egg substance are here so great and their localization so precise that the figures and descriptions of these might well seem to be exaggerated. I therefore welcome the opportunity of publishing a series of photomicrographs of these eggs, an opportunity which I owe to the skill and courtesy of Misses Foot and Strobell. Their method of photomicrography, which they have fully described in previous

publications (Foot and Strobell, 1899, 1900, 1901, 1902) is, in some respects, the most satisfactory ever devised and yet it is as simple as it is complete. The distinguishing feature of this method consists in the accuracy and rapidity with which an exact focus can be obtained; this fact will be better appreciated when the photographs which illustrate this paper are examined in detail and when it is said that thirty such photographs, all of them satisfactory, were taken in less than four hours. With more time and material a more complete series of stages might have been photographed, but this series is sufficiently complete to show the principal features in the differentiation and localization of the egg substances.

All of the photographs are of living eggs in sea water and were taken with a Zeiss Apochromat Obj. 16 mm., Compensating Oc. 4, the bellows length being sufficient to make the magnification 112 diameters. The photographs have not been reduced in the process of reproduction and *neither the negatives nor prints were retouched or altered in any respect whatever.*

The eggs photographed were artificially fertilized and the earliest stage shown (Photo 1) was taken about three quarters of an hour after the sperm were mixed with the ova, but not more than fifteen minutes after the entrance of the spermatozoön into this particular egg. The method of procedure was to place a large drop of sea water containing a considerable number of eggs on a slide and cover with a glass supported by glass "feet" 170 μ thick (the eggs are about 150 μ in diameter). Suitable eggs were then selected and photographed by daylight, the exposure ranging from ten seconds to one minute; the shorter exposure was found to be sufficient while the longer was greatly overtimed (Photos 1 and 13). In order to obtain good color values it is necessary to have the diaphragm rather widely open and this renders the definition somewhat less distinct than it otherwise would be. Furthermore a low magnification was necessary in order to secure sufficient depth of focus to show the entire egg; even with the power employed it was not possible to bring the whole of the egg into good focus at one time. In spite of these evident disadvantages the photographs are really remarkable. Few, if any, other eggs are known in which the organization is so notable as in *Cynthia*

and certainly nothing like this has ever before been photographed in the living condition. I wish here to express my great indebtedness to Misses Foot and Strobell for their kindness in making the photographs and also in superintending the production of the plate.

Differentiations and Localizations of the Oöplasm.

In the ovarian egg of *Cynthia* there is a peripheral layer of protoplasm, free from yolk, in which the "test cells" are imbedded and which contains numerous orange-yellow pigment granules which are uniformly distributed. The central portion of the egg consists of yolk-laden protoplasm which is slate-gray in color and lying somewhat eccentrically within the egg is the large, clear germinal vesicle. Before the egg leaves the ovary the chorion is formed and the "test cells" are extruded into the space between the egg and the chorion.

After the egg is laid the germinal vesicle breaks down, but the polar bodies are not formed until after the egg is fertilized. If fertilization does not take place the polar bodies are never extruded, and the clear, the yellow and the gray substances remain in the positions in which they were before the wall of the germinal vesicle disappears. If the egg is fertilized, however, a most astonishing series of movements occur which lead to the localization of the different oöplasmic substances in definite regions of the egg.

The spermatozoön always enters the egg near the vegetal pole and immediately after its entrance the peripheral layer of yellow protoplasm flows rapidly to this pole where it collects as a cap[1] (Photos 1 and 2). At the same time the clear substance derived from the germinal vesicle also flows to the lower pole where it lies between the cap of yellow protoplasm below and the yolk above. This streaming of protoplasm to the lower pole takes place so rapidly that its movements can be directly observed. Within ten or fifteen minutes after the entrance of the sperm into the egg all of the clear and yellow protoplasm has collected at the lower pole while the opposite pole where the polar bodies

[1] In all the photographs the yellow substance appears very dark, the gray substance less dark while the clear protoplasm is relatively light.

are formed is at this stage rich in yolk and is slate-gray in color.

After the clear and the yellow substances have collected at the lower pole the sperm nucleus and aster move toward one side of the egg which future development shows to be the posterior pole; the clear and yellow substances are also drawn over to this side of the egg and in such a manner that the yellow cap is transformed into a superficial band or crescent which lies just below the equator of the egg on the posterior side, its arms extending forward on each side about half way around the egg (Photos 3 and 4). Owing, perhaps, to the way in which this crescent is formed from the cap of yellow protoplasm its ventral border is sharper and its substance is of a deeper yellow than the dorsal border. At the middle of the yellow crescent is a small area of clear protoplasm which first gathers around the sperm as it enters the egg and which afterward lies at the middle of the crescent throughout the entire development; this clear protoplasm is seen in profile at the middle of the crescent in Photo 3.

The movement of the protoplasm to the posterior pole is apparently initiated by the movement of the sperm nucleus and aster to this pole; here the sperm aster divides giving rise to the amphiaster and here the two germ nuclei meet. The axis of elongation of the amphiaster is always at right angles to the axis which connects the animal and vegetal poles while its middle lies in the plane of the first cleavage and in the median plane of the embryo. The amphiaster lies beneath the yellow crescent and some distance from the surface of the egg and the long axes of the two coincide. In Photo 4 the clear line in the middle of the crescent is the amphiaster seen through the superficial layer of the crescent. It is probable that there is some causal connection between the elongation of the amphiaster and the formation of the crescent.

The clear protoplasm which also moves to the posterior pole along with the yellow is chiefly withdrawn from the surface and aggregated around the sperm nucleus and aster, though a portion of it comes to the surface just above (ventral to) the crescent (Photo 3). As the time for the first cleavage approaches the

germ nuclei and amphiaster move to the center of the egg and the clear protoplasm goes with them. Finally in the telophase of the first cleavage the clear protoplasm moves into the upper (animal) hemisphere, while the yellow and gray protoplasm are situated in the lower (vegetal) hemisphere.

The first cleavage cuts through the middle of the crescent, the clear protoplasm, and the yolk, the division of all the egg substances being bilaterally symmetrical. *At the close of this cleavage each of these substances occupies its definitive position in the egg (Photos 6–8). The upper clear half of the egg gives rise to ectoderm; the crescent of yellow protoplasm surrounds the posterior side of the egg just below the equator and is later transformed into the muscle and mesenchyme cells of the larva; the gray protoplasm occupies the remainder of the lower hemisphere and gives rise to the endoderm, to the chorda, and to the neural-plate.* Two areas are distinguishable in the gray substance, though I had failed to see them until my attention was called to them by the photographs; the posterior part of the gray material lying in front of the crescent and extending some distance anterior to the vegetal pole is deeper in color and contains more yolk than the anterior portion; the latter forms a light gray crescent around the anterior border of the vegetal hemisphere, just as the yellow protoplasm forms the yellow crescent around its posterior border. The 2-cell stage when seen from the right or left sides (right side in Photo 8) shows all of these areas distinctly, the yellow crescent at the posterior pole (very dark in the photograph), the deep gray material anterior to this, and the light gray crescent occupying the anterior third of the vegetal hemisphere and extending a little above the equator, while the clear protoplasm is located chiefly in the upper hemisphere. The dark gray portion of the vegetal hemisphere gives rise to the endoderm of the larva, the light gray crescent to the notochord and neural plate.[1] *All the principal organs of the larva in their definitive positions and proportions are here marked out in the 2-cell stage by distinct kinds of protoplasm!*

[1] It is an interesting question whether the chorda-neural-plate crescent of the ascidian egg corresponds to the "gray crescent" of the frog's egg. In both cases it lies on the anterior side of the egg and in the vicinity of the dorsal lip of the blastopore, but it is not positively known that chorda and neural plate are derived from it in the frog, as is the case in the ascidian, though this seems probable.

Although these different oöplasmic substances are chiefly localized in certain regions of the egg, which give rise to certain portions of the embryo, this segregation is not quite complete. Most of the clear protoplasm is found in the upper (ectodermal) half of the egg but some of it is also present in the lower half. Most of the yolk is found in the lower (endodermal) half of the egg, but a little of it is found in the upper half. Almost all of the yellow protoplasm is located in the mesodermal crescent, but a very small amount of it is found around the nuclei of all the cells. Thus samples of all of these egg substances are contained in all of the cells; nevertheless the segregation is so nearly complete that the clear, the gray, the light gray and the yellow areas are marked out with the greatest distinctness (Photos 7, 8).

In the 4-cell stage, as shown by Photos 9 and 10, the distribution of these substances remains as in the 2-cell stage, the yellow crescent being confined to the vegetal hemisphere and the posterior quadrants, the gray crescent to the vegetal hemisphere and the anterior quadrants, while the deep gray, yolk-laden substance lies between these crescents at the vegetal pole and the clear protoplasm occupies most of the animal hemisphere of the egg.

In the 8-cell stage the localization of these substances is the same as in the preceding stages, the clear protoplasm lying above the third cleavage plane and the other substances below it (Photo 11). The perfectly sharp boundaries of the crescent do not coincide with any of the cell boundaries, gray substance being found in the posterior dorsal cells above, below, and anterior to the crescent (Photo 11). The clear notch in the posterior profile of the crescent in Photo 11 is a cap of the same clear protoplasm which gathered around the sperm head at its entrance and afterwards lay at the middle of the crescent (Photo 3). In the 8-cell stage this clear protoplasm takes the form of two caps on the surface of the yellow crescent and adjoining, on each side, the median plane. In this same stage a small amount of yellow protoplasm may be seen around the nuclei of all the cells (Photo 11). This perinuclear yellow substance is most abundant in the posterior-ventral and in the anterior-dorsal cells; in the former it lies chiefly on the dorsal and lateral sides of the nuclei, in the latter on the posterior and lateral sides. In subsequent divisions of these

cells this perinuclear plasm retains these positions and therefore goes into certain daughter cells and not into others. Consequently even in those cells in which it is found very sparingly this yellow protoplasm is localized with great definiteness.

In the 16-cell stage all these different substances remain in exactly the same positions which they held at the 2-, the 4- and the 8-cell stages (Photos 12–14). The eight ventral cells are all similar in appearance and are composed chiefly of clear protoplasm. The yellow crescent still surrounds the posterior half of the dorsal hemisphere, as in the 1-cell stage, but it is now contained in four cells; it occupies the posterior and lateral portions of these cells while the anterior and median portions are composed of gray substance. The boundary between these two substances could not be sharper if they were actually, as at first sight they seem to be, separated by a cell wall (Photo 14); only at the next stage, however, are these substances segregated into separate cells. The gray crescent occupies the anterior portions of the four anterior cells of the dorsal hemisphere, the posterior portions of these cells being composed of deep gray (endodermal) substance (Photo 14).

At the 32-cell stage (Photos 15, 16) the substances of the yellow and the gray crescents are finally segregated into separate cells, although a small portion of deep gray substance is still contained in the median cells of the yellow crescent; however this gray material moves in from the surface so that it does not show in photographs of the entire egg. The yellow crescent now consists of six cells, four median ones which are small and one pair of lateral ones which are relatively large (Photos 15, 16). The gray crescent consists of four cells of equal size and similar constitution. The ventral half of each cell is clear, contains little yolk and gives rise to most of the neural plate, the dorsal half is light gray in color, is yolk-laden and gives rise to the chorda. From this stage onward the ventral hemisphere is composed of clear cells, all of which are much alike; for this reason no photographs are given of the later stages of these cells.

In subsequent stages all of the cells of the gray and yellow crescents divide in a vertical direction (parallel to the egg axis) these divisions occur first in the gray crescent and in the most

anterior pair of cells of the yellow, then in the pair of yellow cells adjoining the latter behind, and finally in the posterior median pair. The subdivision of the cells of the gray crescent occurs at the 44-cell stage; the ventral product in each case is a small clear cell which ultimately forms the posterior portion of the neural plate, the dorsal products are larger and are gray in color and ultimately develop into the chorda.

In the case of the yellow crescent both products of the posterior median cells give rise to mesenchyme, but the ventral ones contain those caps of clear protoplasm which were visible in the 8-cell stage (Photo 11) and which in a still earlier stage first appeared around the entering spermatozoön (Photo 3). The dorsal products of the other two pairs of crescent cells give rise to mesenchyme, while from the ventral halves come the muscle cells of the tadpole's tail. The mesenchyme cells are clear and faintly yellow in color, the muscle cells are a deep yellow and these two substances are distinguishable in their definitive positions even as early as the 1-cell stage (Photo 3).

Unfortunately no photographs of the early stages of gastrulation were taken. I have however studied and drawn every step of this process both in living and in prepared material. Gastrulation begins with the depression of the endoderm cells just posterior to the chorda cells, and is later continued by the rolling in of cells around the margin of the blastopore. In this manner the neural plate cells come to overlie the chorda cells, and the muscle cells, the mesenchyme.

In the closure of the blastopore the posterior (ventral) lip remains stationary until the last stages of the process, while the anterior (dorsal) lip grows backward over the gastrocoel until the blastopore is reduced to a longitudinal groove between the muscle cells of each side (Photo 17). In the overgrowth of the dorsal lip the rows of muscle cells as well as the blastopore groove are forced to the hinder end of the embryo and the muscle rows are tilted up at their anterior ends until they are transverse to the long axis (Photo 17). Later the ventral lip overgrows the remnant of the blastopore and the ectoderm of this lip forms a pair of V-shaped folds which fuse from behind forward and thus cover the dorsal lip and roll the neural plate up into a tube. In

this overgrowth of the ventral lip the transverse rows of muscle cells again assume an antero-posterior direction in the embryo (Photos 18–21).

Since the neural plate is composed of relatively transparent cells which overlie the gray chorda plate, it is not well shown in the photographs, unless seen in profile (Photos 22, 23). In the dorsal view shown in Photo 18 seven or eight transverse rows of cells may be indistinctly seen in the neural plate. The chorda plate, which contains considerable yolk and is gray in color lies under the neural plate. It consists at first of a single transverse row of eight cells, then by the division of these cells two such rows are formed and finally by shoving together from the sides this plate becomes much narrower and longer. In later stages the chorda plate and the neural plate push back between the muscle cells of each side until they reach the hinder end of the embryo, thus establishing the characteristic appearance of the young larva shown in Photo 21. It can now be seen distinctly that the deep yellow cells have become the lateral muscles in the tail, that the light gray cells of the chorda plate have formed the fusiform chorda which lies between the muscle cells, and that the deep gray cells form the gastral endoderm. At the posterior end of the chorda is a group of light yellow cells which connect the muscle rows of the right and left sides; these are the caudal mesenchyme cells. At the anterior ends of the muscle rows are the clear areas of the trunk mesenchyme, which are also of a faint yellow color, while around the entire embryo is a clear layer of ectoderm cells (Photo 21).

The form of the larva is now well established and subsequent development changes this form only in minor features. In Photo 23 the tail is much elongated and bent toward the ventral side. Three rows of muscle cells with clear nuclei can be seen on the left side of the tail, while the larva is tilted toward the left so that the dorsal row of muscle cells of the right side is also visible; with the elongation of the tail the individual muscle cells have become much longer than in previous stages. Between the dorsal rows of muscle cells of the right and left sides is a clear line which is the neural tube; anteriorly this tube overlies the dark gray endoderm and hence it is not clearly visible in the

photograph, but its anterior end appears as a clear, triangular area, notched where the tube is still open at the anterior end of the larva. This clear, transparent condition of the nervous system, both in the tail and trunk regions shows that the yellow protoplasm does not enter into its formation and that the muscle cells are not "neuro-muscular" cells as claimed by Castle (1896). In this and the following photograph (Photo 24) the larva is well developed, though the sense organs have not yet appeared in the sense vesicle. The most important organs of the larva are here clearly recognizable in the photographs of the living tadpoles, viz., the muscles, the notochord, the central nervous system, the gastral endoderm, the caudal and trunk mesenchyme and the ectoderm. *The substance of each of these organs is peculiar in color and constitution and these different substances may all be traced back to the 2-cell stage, where they occupy positions corresponding to their ultimate locations in the larva, while the substances of the ectoderm mesoderm and endoderm are recognizable in the unsegmented egg.* With the exception of the early gastrula stages, which were not photographed, every important step in the transformation of these substances into the organs named can be followed in the photographs of the living eggs and embryos!

Nature and Potency of the Oöplasmic Substances.

The fact that definite blastomeres of the ascidian egg give rise to definite portions of the larva has long been known (Van Beneden and Julin, 1884; Castle, 1896). Furthermore Chabry (1887) found that when certain blastomeres were killed the remaining ones gave rise only to a partial larva. On the other hand, Driesch (1895, 1903) and Crampton (1897) found that individual blastomeres of the ascidian egg developed into entire larvæ. The mere observation of the egg of *Cynthia* shows that certain areas are marked out from the time of fertilization, or even earlier, by distinct kinds of protoplasm and that these areas give rise in the course of normal development to definite organs. But, in view of the work of Driesch and Crampton, by what right are these areas called organ-forming regions and what is the justification for calling the substances of these areas organ-

forming substances? The answer to both of these questions is the same, viz., in the absence of a region or substance, the organ to which it would normally give rise is not produced; and conversely each substance develops, if it develops at all, into the parts which it would normally produce. Experiments which I have carried out on ascidian eggs[1] show that the development of isolated blastomeres is strictly partial, as was first shown by Chabry and afterwards denied by Driesch. As yet I have been unable to get the isolated substances of the unsegmented egg to develop at all, but when they are isolated during the cleavage stages they develop only into the parts which they would normally produce, while the portions of the egg or embryo which lack these substances develop into embryos which lack the corresponding organs. Since the first cleavage of the egg is bilaterally symmetrical and divides all the substances of the egg equally, each of the first two blastomeres contains one half of all the organ-forming regions and substances; and since isolated blastomeres of the ascidian egg always produce rounded masses of cells which tend to close over the injured surface, many of these half embryos have the appearance of whole embryos of half size: but a careful study of living material as well as of stained preparations and sections shows that the larvæ are still incomplete up to the time of the metamorphosis. When the division of the egg or embryo is made along any other plane than the median one nothing even remotely resembling a normal larva is obtained. Every substance of the egg develops, if it develops at all, into the organs which it would normally produce, and while it has not been possible to isolate these substances in the unsegmented egg, their appearance is the same before and after cleavage begins and under these circumstances there is small room for doubting that even in the unsegmented egg these are actually organ-forming substances.

Therefore in the unsegmented egg and early cleavage stages of *Cynthia partita* we have the most complete differentiation and localization of the oöplasm ever yet reported for any egg. Apart from the nuclei, the centrosomes and the asters, there are visible in the 2-cell stage six different kinds of cytoplasmic substance,

[1] These experiments will be published in full elsewhere.

each of which gives rise to some specific portion of the larva and is here present in its definitive position and proportions, viz., the clear protoplasm which gives rise to the ectoderm, the gray substance which produces endoderm, the deep yellow substance which develops into the muscle cells, the light yellow which goes into the mesenchyme, the clear protoplasm at the middle of the yellow crescent which becomes caudal mesenchyme and the light gray substance of the gray crescent which gives rise to chorda and neural plate. Inasmuch as it is difficult to refer to these different substances of the egg by the purely descriptive terms which have been employed thus far, I propose to designate them by names suggestive of the parts to which they ultimately give rise, viz., ectoplasm, endoplasm, myoplasm, chymoplasm, caudal chymoplasm and chorda-neuroplasm.[1] Of all of these substances the mesoplasm (myoplasm and chymoplasm) alone takes its definitive position before the first cleavage; the other substances reach their final positions only at the close of this cleavage. But although the localization is not complete in the unsegmented egg the ectoplasm and endoplasm are nevertheless clearly differentiated before cleavage begins; I am unable to say whether the chorda-neuroplasm is also differentiated at this stage.

Cleavage and Germinal Localization.

In the early stages of development it is apparent that the cleavage planes do not closely follow the lines of separation between the different substances of the egg. The yellow crescent is bisected at the first cleavage; the second cleavage passes anterior to it; the third cleavage plane lies some distance above (ventral to) the upper border of the crescent; the fourth cleavage bisects the halves of the crescent on each side of the median plane. No one of these first four cleavage planes follows any one of the boundaries of the crescent; the same is also true of all the other oöplasmic substances. Although the localization of these substances is precise and definite, the localization pattern does not correspond to the early cleavage pattern. In the later cleavages some of the division walls do closely correspond with the planes

[1] The substances of the chorda and neural plate are not clearly distinguishable from each other before the 8-cell or 16-cell stage.

of separation between these substances so that these various substances are ultimately segregated into definite cells, but this perfectly definite type of localization arises without reference to cell division and is not appreciably altered by subsequent divisions. During the first cleavage the yellow crescent substance may be seen to be undergoing complex vortical movements, but this does not permanently change the form or position of the crescent. In the unsegmented egg and in all the subsequent stages of the cleavage the yellow crescent occupies its initial position on the posterior side of the egg below the equator, irrespective of the position of the cleavage planes. Likewise the gray crescent, the ectoplasm and the endoplasm occupy the same positions in the egg at the beginning of gastrulation as in the 2-cell stage; in fact so far as localization is concerned the condition at the close of cleavage is the same as at its beginning.

Cytoplasmic and Nuclear Organization.

All of these different organ-forming substances are present, and are shown in the photographs, as early as the close of the first cleavage, some of them much earlier. In fact the clear ectoplasm, the gray endoplasm and the yellow mesoplasm are recognizable in the ovarian egg. Here the mesoplasm forms a peripheral layer around the whole egg in which the "test cells" are imbedded, the ectoplasm is contained within the large germinal vesicle, while the endoplasm occupies the remainder of the cell. Tracing these differentiations still further back it is found that at least a portion of the mesoplasm comes from the sphere substance (archoplasm), which is probably derived in part from the nucleus of the last oögonic division (Conklin, 1902). The yolk also is formed by the activity of the "yolk matrix" (Crampton, 1899) or yolk nucleus which is probably derived from the sphere substance. Portions of the ectoplasm, mesoplasm and endoplasm [1] are thus

[1] It may of course be objected that the yellow, the gray, and the clear substances of the ovarian egg have not been proven to be differentiated for particular ends and this I freely grant to be the case. Furthermore I do not see how this question could be tested experimentally, especially in the case of the immature ovarian egg. The fact that these substances are visibly different from one another in the oöcyte and that in all respects they resemble the ectoplasm, mesoplasm and endoplasm of the cleavage stages, to which they ultimately give rise, is the only reason for continuing to call them by these names in this earlier stage.

derived from the nucleus, the first from the nucleus of the oöcyte, the last two from the nucleus at the last oögonic division.

This remarkable condition in which considerable portions of the oöplasm can be traced back to the nucleus is of the greatest theoretical importance. From all sides the evidence has accumulated that the chromosomes are the principal seat of the inheritance material until now this theory practically amounts to a demonstration. On the other hand all persons who have much studied cell-lineage have been impressed with the fact that polarity, symmetry, differentiation and localization are first visible in the cytoplasm and that the positions and proportions of embryonic parts are dependent upon the location and size of certain blastomeres or cytoplasmic areas. However in the fact that large quantities of "nuclear sap" containing dissolved oxychromatin escape into the cell body at every mitosis (*v.* Conklin, 1902) and that these nuclear substances then contribute to the formation of specific organ-forming substances of the cytoplasm we see a possible means of harmonizing the facts of cytoplasmic organization with the nuclear inheritance theory.

Types of Germinal Organization.

By those who maintain the view that the egg is typically composed of "simple undifferentiated protoplasm" the remarkable organization of the ascidian egg will probably be regarded as an extreme case of precocious differentiation. This may perhaps be the case but the fact that germinal differentiations and localizations occur in the eggs of annelids, mollusks, nemerteans, echinoderms, ctenophores, nematodes and ascidians shows that it is by no means a rare phenomenon and it really seems as if the burden of proof were shifted to those who maintain that the egg is typically undifferentiated. Unquestionably the egg is less highly differentiated than the embryo or larva, as organ-forming substances are simpler than the organs to which they give rise, but the evidence drawn both from observation and experiment shows conclusively that in a large number of animals the substances of the egg are not homogeneous nor equipotential. But even granting that there are cases in which there is no such differentiation of the oöplasm, this supposed lack of differentiation can apply only to

portions of the egg, the cytoplasm for instance, for of course there must be determinative factors ("determinants") somewhere in the ovum, probably in the nucleus which differ from one another in kind.

However in the phyla named the localization of morphogenic substances in the cytoplasm is sufficiently definite to warrant a comparison of one group with another. In all the ascidians which I have studied and apparently in all which have been studied hitherto, the type of localization is the same. Furthermore there is good reason for supposing that this type is essentially like that of *Amphioxus* and Amphibia (on this subject see Conklin, 1905). Judging from the work which has been done on the organization of the egg in other phyla, this chordate type is very distinct from that of annelids, mollusks, nemerteans, echinoderms, nematodes or ctenophores. In fact it seems necessary to recognize several distinct types of localization.

If one has regard only to the localization of the substances of the germinal layers there is considerable uniformity among most metazoa in their pregastrular stages. In almost all cases the ectodermal substances are localized in that hemisphere of the egg which is nearest the polar bodies, and in this the ascidians are no exception to the rule; in many cases the mesodermal substances are at first localized at the opposite pole, though only among the echinoderms (*Strongylocentrotus*, Boveri, 1901) is this localization persistent; among annelids, mollusks, and ascidians the mesodermal substances early move from this pole to the posterior side of the egg.

However in the localization of specific organ bases there are many notable differences among these phyla. In this regard the annelids and mollusks and perhaps the nemerteans belong to one type, the chordates, nematodes and ctenophores to entirely different types; in fact the localization of organ bases in the ascidian egg does not resemble that in the other phyla named any more closely than does the localization of the larval or adult organs of these phyla; indeed, the principal chordate features are already represented in the ascidian egg by characteristically localized organ bases as early as the 2-cell stage.

Since the time of Cuvier the principal criterion of homology

has been, in the words of Owen, "correspondence in the relative position and connexion of parts." Such correspondence has been found to be much more fundamental than resemblances in size, proportions, or details of structure. Similarly in germinal organization it seems probable that the relative positions and connections of organ bases are essentially alike in different members of a phylum, though in other respects, they may vary widely. A peculiar type of localization of organ bases is thoroughly characteristic of the ascidian egg and probably the same thing is true of other phyla. If this be true, different phyla do not approach one another more closely in the earliest stages of germinal localization than in the cleavage or gastrular stages.

Origin and Evolution of Germinal Organization.

The fact that there are various types of germinal localization corresponding to different types of adult organization will be explained by most persons as due to the gradual "acceleration" of development, or the shifting of adult characters back to earlier and earlier stages of the ontogeny. It is but natural that those whose attention is focused upon adult structures should regard the adult as primary, the germ as secondary; but it is surprising that embryologists also have almost universally held a similar view. Even students of cell-lineage and of the organization of the egg have generally regarded this organization as secondary and have explained it as the result of "precocious segregation," or of the "reflection of larval or adult characters back upon the egg."

Such conclusions are not founded upon observation nor experiment but upon preconceived notions as to the importance of the adult and the extreme simplicity of the germ. The whole life cycle is commonly viewed from the standpoint of the adult, and all other stages are supposed to exist *for the purpose* of leading up to a definite end stage. Similarly evolution is looked upon as the transmutation of definite end stages into others by direct modification of certain adult structures, which in some way or other modify the germ and thus become inherited.

But what is the evidence that, in either ontogeny or phylogeny, the adult is primary and the germ secondary? What is the

ground for supposing that evolutionary changes first occur in the end stages and only later affect the earlier stages in the life cycle? In spite of the age-long controversy as to the inheritance or non-inheritance of acquired characters there is no satisfactory evidence that particular modifications of any adult part ever produce specific modifications of the germ. All the evidence available seems to show that the soma stands to the germ in the relation of environment and that the only influence exercised by the former upon the latter is of a general character, as Weismann has so ably argued.[1] Furthermore the difficulty of conceiving of any method by which adult characters might be transferred to the germ is well known. No hypothesis ever yet proposed for the solution of this problem harmonizes with the established facts of oögenesis and spermatogenesis. If such transfer occurs, of which there is no sufficient evidence, it can only take place by methods of which we are at present wholly ignorant.

On the other hand there is much to be said in favor of the view that the germ is primary, the adult secondary and that heritable modifications first arise in the germ and only later appear in the adult. Apart from the fact that the germ gives rise to the adult and to other germs, it is known that in certain cases apparently slight modifications of the germ may produce profound modifications of the adult, whereas the reverse is not known to be true. One of the most convincing evidences of the truth of this view is found in cases of cross breeding, particularly in hybridization, where it is certain that hybrid characters of an offspring are directly due to the hybrid character of the germ, since they can have no other possible cause. The evidence drawn from experiments on eggs and embryos on first thought seems to be conflicting; in some cases fragments of eggs or embryos give rise only to partial larvæ and injured eggs produce only embryos showing more or less serious defects; in other cases entire embryos are produced under these conditions, but these results cannot be regarded as destructive of this argument for, as has long been maintained by Roux, such cases of entire

[1] Undoubtedly one important cause of germinal variation is to be found in the influence of changing environment upon the germ, but this is far from saying that particular modifications of the adult are transmitted to the germ.

development of egg fragments may be the result of regenerative, or regulative, processes. It is not usually possible to connect definite modifications of the adult with definite alterations of the germ from which it developed, but one remarkable instance in which this is possible is found in cases of inverse symmetry. In sinistral gasteropods, and presumably in all other cases of inverse symmetry, the cause of inversion is to be found in the inverse organization of the unsegmented egg and I have elsewhere (1903) shown reason for believing that this may be due to the maturation of the egg at opposite poles in dextral and sinistral forms. Here one of the most sudden and profound alterations of structure with which we are acquainted may be traced back to a specific modification of the germ.

These facts point to the conclusion that the complex organization of an egg, such as that of an ascidian, has not arisen through the "reflection of adult characters upon the egg," but rather that this organization is primary. Furthermore they seem to indicate that evolution has taken place, not through modifications of adult structure, but through changes in germinal organization; modifications of this organization, however produced, are probably the real causes of evolution.

This conclusion, which has grown out of a study of the complex organization of the germ and its relation to adult organization, harmonizes entirely with the mutation theory of DeVries; it indicates how mutations in elementary germinal characters may appear as widespread modifications in the mature organism; it offers an explanation of otherwise inexplicable variations of adult structure, such as inverse symmetry; and finally it suggests a possible solution of that vexed problem of the origin of phyla, not by the transmutation of one adult form into another, as is assumed in all previous hypotheses, but by relatively simple alterations of the type of germinal organization.

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December 30, 1904.

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DESCRIPTION OF PHOTOGRAPHS. (PLATE XI.)

All the photographs are of the living eggs and embryos of *Cynthia* (*Styela*) *partita* in sea water and are magnified 112 diameters. Prints from the original negatives were arranged in order and photographed and the plate is an actual print (Rotograph Process) from this negative. These photographs are not diagrams but like the specimens themselves they require and will repay careful study. That portion of the egg which appears darkest in the photographs is the orange-yellow mesoplasm, that which is lightest is the transparent ectoplasm, while that which is apparently intermediate in shade between these two is the slate-gray endoplasm. The contrast between these substances is therefore greater in reality than appears in the photographs. Every egg or embryo is inclosed in a transparent chorion which does not show in the photographs; within the chorion and around the periphery of the egg are numerous "test cells" which contain yellow pigment.

PHOTO 1. Egg about fifteen minutes after the entrance of the sperm showing the yellow protoplasm as a dark cap at the lower pole where the sperm lies. The clear protoplasm is the light zone above the yellow; the rest of the egg is gray.

PHOTO 2. Egg about twenty minutes after the entrance of the sperm; the egg substances are shown as in the preceding photograph.

PHOTO 3. Egg about thirty minutes after the entrance of the sperm, seen from the right side. The yellow protoplasm is moving to the posterior pole and forming the crescent there; the clear protoplasm lies chiefly above (ventral to) the crescent; in the middle of the crescent and at the periphery of the egg is a small spot of clear protoplasm (caudal chymoplasm) which first appears around the entering sperm and ultimately goes into a pair of caudal mesenchyme cells.

PHOTO 4. Egg about thirty-five minutes after the entrance of the sperm, showing the yellow crescent as a dark band with a clear area through its center: the latter is the first cleavage spindle. Both the crescent and the periphery of the egg show a slight notch in the lower (ventral) border, which is the beginning of the first cleavage furrow. The egg is somewhat obscured by overlying test-cells which here and elsewhere give it a mottled appearance; high focus.

PHOTO 5. Egg about forty minutes after the entrance of the sperm, viewed from the posterior-ventral pole. The cleavage furrow is deepest in the region of the crescent. Above the crescent is the clear (ventral) ectoplasm. High focus.

PHOTO 6. Egg about forty-five minutes after the entrance of the sperm. Two-cell stage viewed from the animal pole showing the yellow crescent at the posterior margin of the egg.

PHOTO 7. Stage similar to the preceding, viewed from the posterior pole; below the crescent is shown the gray endoplasm of the vetegal pole, above the crescent the clear ectoplasm of the animal hemisphere, in the furrow at the middle of the crescent a small amount of clear chymoplasm.

PHOTO 8. Stage similar to the preceding, viewed from the right side (an end view of one of two cells), showing the clear ectoplasm in the upper (animal) hemisphere, the yellow crescent (mesoplasm) at the posterior pole, the light gray crescent (chorda-neuroplasm) at the anterior pole and the dark gray endoplasm between the two crescents at the lower pole. The definitive localization of these substance is complete at this stage.

PHOTO 9. Four-cell stage from vegetal pole, showing the yellow crescent across the two posterior cells. The anterior cells lie at a lower level than the posterior ones and the focus is such that only the ends of the crescent show clearly.

PHOTO 10. Four-cell stage from the animal pole, high focus; many test cells cover the egg; the yellow crescent, which lies on the lower side of the posterior cells, shows indistinctly through the egg; an area of clear protoplasm is shown in each of the cells.

PHOTO 11. Eight-cell stage from the right side; the upper cells contain the clear ectoplasm, though a small amount of yolk is found at the periphery of each cell; most of the yellow protoplasm is contained in the yellow crescent, the outline of which is very distinct, but a small amount of yellow protoplasm is found around the nuclei of all the cells; in the posterior ventral (upper) cells this lies on the lateral and dorsal side of the nucleus, in the anterior dorsal (lower) cells it lies on the lateral and posterior side of the nucleus. At the middle of the yellow crescent and seen as a notch in its posterior outline is a small cap of caudal chymoplasm (the same as that seen in Photo 3). The yellow crescent is bounded by dark gray endoplasm which extends forward to the middle of the anterior-dorsal cells; the gray crescent of chorda-neuroplasm occupies the anterior portions of these cells. The forward slant of the vertical (second cleavage) furrow and the "cross furrow" formed by it and the third cleavage are clearly shown. Photos 3, 8, and 11 are all viewed from the right side and the localizations of the same organ-forming substances in the 1-, 2- and 8-cell stages are clearly shown in these photos.

PHOTO 12. Sixteen-cell stage from dorsal side, view slightly oblique. The eight dorsal cells are clearly shown, while three transparent ventral cells are indistinctly shown on the left-anterior periphery. The yellow crescent is contained in the four posterior cells, the lighter margins of the four anterior cells represent the gray crescent. The median cells behind are nearly filled with yellow mesoplasm, save for the clear nuclei and a small wedge of gray substance in the anterior portion of these cells; the lateral portions of the cells just anterior to these are composed of yellow substance, their median portions of gray material.

PHOTO 13. Sixteen-cell stage from the posterior pole, showing the four yellow crescent cells with clear nuclei; below them is the gray endoplasm, above them the clear ectoplasm; the ectoderm cells are indistinctly shown with a trace of yellow substance around nuclei of the four posterior cells.

PHOTO 14. Sixteen-cell stage transitional to 32-cell stage, dorsal view showing eight cells. The localization of the different oöplasmic substances is the same as in Photo 12, but the focus is a little deeper. The yellow and gray crescents are remarkably distinct; between the two is the area of deep gray endoplasm; the light area on the inner border of the yellow crescent is chymoplasm.

PHOTO 15. Thirty-two cell stage, dorsal view. The small posterior crescent cells have divided transversely, forming four small cells; the large mixed cell anterior to these is just cutting off its outer yellow portion from its inner gray one. The four anterior cells have divided in an antero-posterior direction thus separating the gray crescent of chorda-neuroplasm from the endoplasm. The nuclei in all the cells appear as clear areas.

PHOTO 16. Thirty-two cell stage, dorsal view, similar to the preceding.

PHOTO 17. Advanced gastrula, posterior view, superficial focus. The blastopore groove is a narrow slit bounded on each side by four large muscle cells, which are derived from the yellow crescent; the lighter colored cells at the bottom of the groove are mesenchyme and are derived from the middle part of the crescent. The dorsal lip of the blastopore (not clearly shown in the photo) closes the groove dorsally and anteriorly.

PHOTO 18. Late gastrula, dorsal view, superficial focus. The U-shaped group of mesoderm cells (Photo 17) is seen from the open end of the U; the ectoderm overgrowing the mesoderm is seen as a light area posterior to the yellow cells; the light wedge-shaped area in the mid-line is the beginning of the neural groove; the apex of the wedge lies between the limbs of the U and marks the point at which the blastopore closes and also the posterior limit of the neural plate. The ectoderm can be seen as a zone of clear cells with transparent nuclei around the periphery and faint indications of these cells with their clear nuclei can be seen forming seven or eight transverse rows of cells across the embryo anterior to the blastopore (the neural plate). The gray endoderm seen through these ectoderm cells gives a dark appearance to all the embryo save the periphery.

PHOTO 19. Late gastrula, dorsal view, deep focus. This stage is later than the preceding and the embryo is tilted slightly toward the right side, so that the plane of symmetry is a little to the right of the middle of the photo; the mesoderm cells are no longer transverse to the long axis but are extending in an antero-posterior direction. In front of the mesoderm is a dark area, the endoderm, in which four transverse rows of cells may be indistinctly seen; around the entire periphery is the clear ectoderm. The anterior portion of the embryo is wider and the posterior part narrower than at any previous stage.

PHOTO 20. Young tadpole, ventral view, superficial focus: the larva is slightly tilted to the right so that the ventral mid-line lies to the right of the middle of the photo. Three rows of rounded muscle cells (six or seven cells in a row), with clear nuclei, lie on each side of the mid line. In front of the muscle cells on each side is a clear area of mesenchyme. The strand of caudal endoderm cells shows in the mid-line between the muscle rows of each side; anterior to the muscle and mesenchyme is the gastral endoderm.

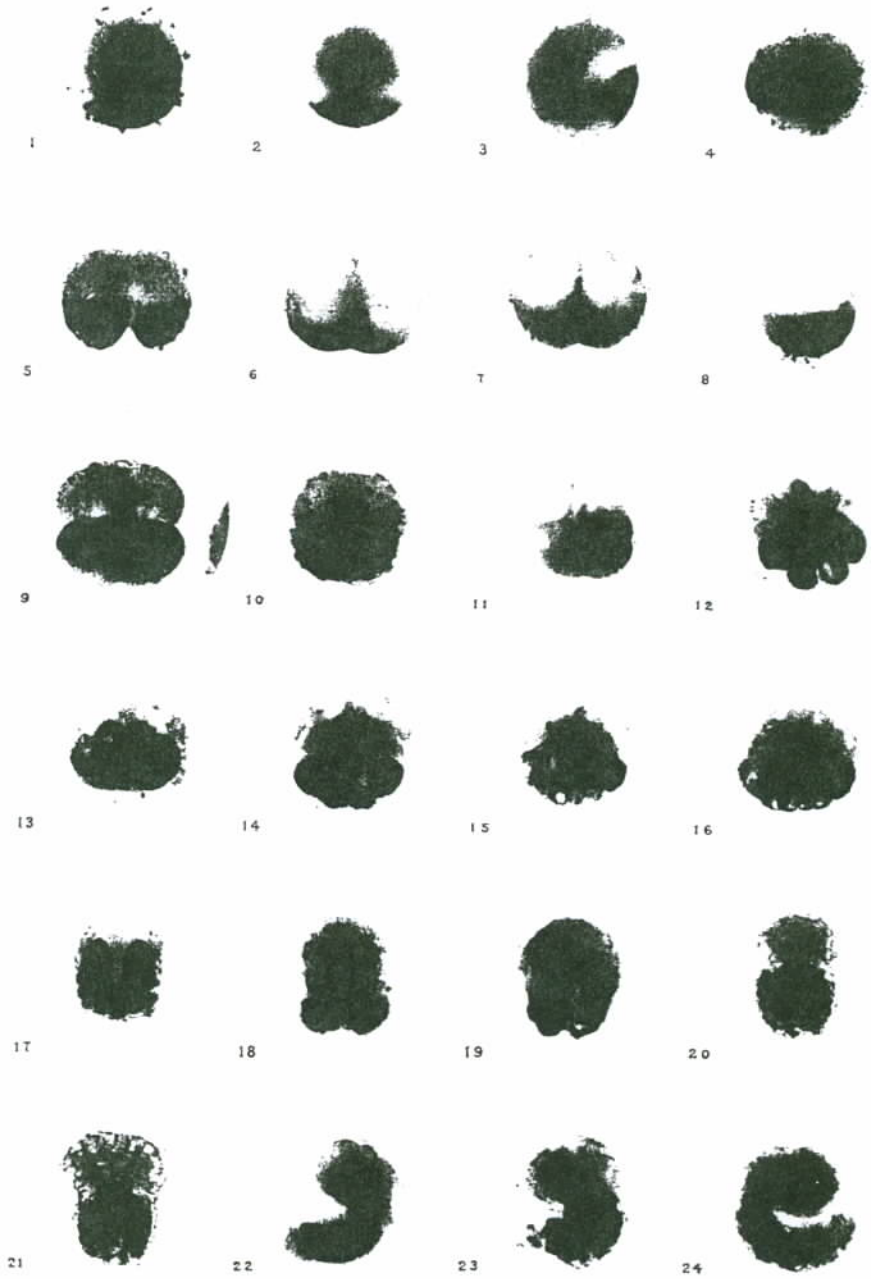
PHOTO 21. Young tadpole, ventral view, deep focus. Between the muscle cells is the fusiform notocord which is composed of wedge-shaped cells. Six muscle cells are visible on each side of the chorda and there are several lighter colored mesenchyme cells at its posterior end. At the anterior ends of the muscle rows is a clear area of mesenchyme cells in which the peribranchial pouches appear. Cells of the gastral endoderm are clearly visible; around the entire periphery are clear ectoderm cells.

PHOTO 22. Young tadpole from left side. The three rows of muscle cells, each with a clear nucleus are faintly shown in the dark area in the tail, the gastral endoderm also appears as a dark area in the trunk, but not so dark as the muscle cells. Around the entire periphery is the clear ectoderm; on the dorsal (convex) side this is especially thick and comprises the neural tube. The hazy areas ventral to the embryo are due to aggregated test cells.

PHOTO 23. Tadpole of about the same stage as the preceding, left-dorsal aspect.

The rows of muscle cells show clearly along the left side ; the dorsalmost row of the right side also shows and between these two is a clear line, the nerve tube (or cord); anteriorly this cord leads to the wedge-shaped light area at the anterior border of the larva, which is the sense vesicle (brain) and which is still open to the exterior. The test cells somewhat obscure the posterior portion of the tail.

PHOTO 24. Tadpole a little older than the preceding viewed from the right side. The tail is much elongated and the muscle cells, each faintly marked by a clear nucleus and dark outline, are also elongated as compared with preceding photos. Two rows of muscle cells are distinctly visible, the third also comes into view at the hinder end of the tail. There are seven or eight cells in each row as indicated by the number of nuclei. The gastral endoderm shows as a dark area in the trunk. The neural cord and tube is the clear area on the dorsal side of the trunk, its anterior limit being marked by a flattened contour line where the neural folds are just closing.



CONKLIN, ORGAN-FORMING SUBSTANCES.