[From the Proceedings of the American Association for the Advancement of Science, Vol. xxvii, St. Louis Meeting, August, 1878.]

Changes Preliminary to Cleavage in the Egg of Clepsine.

By Charles O. Whitman, of Newton Highlands, Mass.

[ABSTRACT.]

The embryology of one of the higher animals may be said to embrace the history of two successive periods, viz., the *preembryonic* and the *embryonic*.

The pre-embryonic period includes the growth and maturation of the egg, impregnation, nuclear changes preparatory to cleavage, the cleavage, the formation of a hollow sphere (*Blastula*, Häckel) from the cleavage products, and the transformation of this sphere into a double-walled sac (*Gastrula*, Häckel; *Planula*, Lankester).

The second, or embryonic period, is introduced by the formation of a *primitive stripe*, formed by the concrescence and concomitant thickening of the two halves of the Gastrula-orifice. During this period the parts of the future animal are sketched out and more or less completely developed.

Older embryologists devoted their attention almost exclusively to the embryonic phases of development; it is only within the last seven or eight years that pre-embryonic phenomena have begun to receive the attention they deserve. It is some of these phenomena to which I shall now ask your attention, making the egg of Clepsine the point of departure.

Clepsine marginata, as many of you know, belongs to the leech family. It is a fish parasite, measuring about one inch when fully grown. It is found in ponds or small brooks where the current is gentle or imperceptible, attached to leaves, stones, fallen branches, etc. The eggs of this species vary in number from twenty to two hundred, according to the size of the specimen and the amount of food it has taken, and are generally attached to the leaves of living plants.

This leech, like others of the genus, has the interesting habit of remaining over its eggs till they hatch. At the time of hatching the young are little developed, and entirely helpless. Their protection is very curiously provided for. Soon after hatching they become attached to the ventral side of the parent, and are thus borne about until fully developed. During this time they receive no food from the parent, as shown by the fact that they develope quite as well when separated from the mother; their only food is the nutritive yolk furnished by the egg.

When, at the end of from two to three weeks from the time of hatching, this store of food is exhausted and the young are developed, the parent seeks to attach itself to a fish. This done, the young leave the mother and creep over the fish and take their first meal.

Origin and Growth of the Egg.—The egg of Clepsine, like that of any other animal, is derived from a simple cell. This primary egg-cell is a spherical body inclosed within a thin transparent membrane, and is composed of three concentric parts, viz.—the protoplasmic body, the nucleus, and the nucleolus. The first step in the development of this cell into the proper ovum, is signalized by the accumulation of fine, opaque granules in the body of the cell, around the nucleus. These granular precipitations are white when seen by reflected light, and exhibit a lively tremulous motion when brought into contact with water—the so-called Brownian movement. They multiply rapidly with the growth of the egg, and soon render the entire ovum opaque. When the egg is about half-grown some of these granules begin to expand by a simple process of growth, thus giving rise to the yolk-spheres. The color of the egg, which may be either white, yellow or green, depends upon the color of the yolk-spheres. As these granules and spheres have a secondary origin, as compared with the semi-fluid protoplasm in which they are imbedded, they have been termed by Van Beneden, *deutoplasm*. The origin of the deutoplasmic elements in the bird's egg or in the reptile's egg is, according to the investigations of Gegenbaur, precisely the same as in the egg of Clepsine, the only difference being, that in the former the yolk undergoes a farther differentiation into white and yellow yolk.

The full-grown egg of Clepsine measures about $\cdot 80^{mm}$ — about the size of a small pin-head — the nucleus about $\cdot 08$ to $\cdot 09^{mm}$, and the nucleolus only about $\cdot 01^{mm}$.

Transformation of the germinal vesicle.— Shortly after the maturity of the egg, the nucleus, or germinal vesicle as it is usually called, undergoes a remarkable transformation. The cause of this change is as yet unknown. It is simply known that the membrane of the germinal vesicle dissolves, the nucleolus becomes invisible, and that the whole or a part of the vesicle assumes the form of a spindle, around the two poles of which the protoplasm of the yolk becomes arranged in radial lines. This form of the nucleus, the precise origin of which was first made known by Auerbach, in 1874, was called by him "*karyolytic figure*," but has since been more appropriately termed *amphiaster*, or double star, by a distinguished naturalist of Geneva, Dr. Hermann Fol.

The spindle of the amphiaster, or bistellate figure, is composed of fibres curving outward, and converging to a point in each pole of the amphiaster. These spindle-fibres appear to differ in no essential respect from the radial polar lines; but in the eggs of some other animals and in the eggs of plants, according to Bütschli and Strasburger, they form the most conspicuous part of the figure.

The entire figure bears a most striking resemblance to the picture produced in iron-dust under the influence of a magnet. This resemblance was at once remarked by Leuckart and others who have seen my preparations; but it is not quite perfect in one particular — that no curves appear in the radial polar lines. But it is to be remembered that these phenomena have thus far been studied in microscopic sections of eggs that have been treated with various acids. It is quite possible that such curves do exist in the living egg, and that they become obscured or obliterated by treatment with acids and staining fluids. That we have here an electrical phenomenon is suggested not only by the general appearance of the amphiaster, but also by the deportment of the two poles during the process of cleavage. This amphiastral form of the nucleus is, as we shall see farther on, one stage in the process of self-division. It is a transitional phase by which one nucleus becomes two nuclei. This division of the nucleus generally results in the division of the egg, or the cell. As often as the cell divides, the amphiastral figure recurs. It is plain, therefore, that we have here a life-phenomenon of very great importance. It is a phenomenon of the deepest interest to the biological student, inasmuch as it stands at the very threshold of all organic life. It is common not only to all eggs, whether the eggs of plants or of animals, but also to all cells, whether embryonic cells or the cells of fully developed tissues. Its universality testifies to the unity of life.

Quiescent state.— After the formation of the amphiaster, the egg appears to maintain a quiescent condition, until, at the time of deposit, it is brought in contact with water. The proof of this fact was obtained in the following manner. I disturbed a leech that was laying its eggs, and, in consequence of the interruption, three eggs were retained in the ovary. The leech manifested no disposition to part with the remaining three eggs. At the end of forty-eight hours I cut the leech through the middle and thus liberated three eggs. To my surprise, I found them in precisely the same condition in which I had found the others two days before. The eggs were kept in both cases and developed in the normal manner. I have observed cases in which the eggs have been retained in the ovary four to five days after they were ripe for deposit. I am the more certain on this point, as I have been able to recognize this time with sufficient accuracy to exclude failure in every case where I made the experiment of cutting the leech to obtain eggs.

This quiescent condition, which can be maintained for at least two days in the egg of Clepsine, and which in the egg of the hen, according to Colasanti, may continue three weeks, and, in rare cases, even four weeks, without fatal injury, reminds one of the analogous fact that seeds can be preserved for years in a similar condition.

Polar Figure.— Soon after deposit, the amphiaster moves towards the periphery of the egg in such a manner that one of its poles comes to lie exactly in the surface. This pole appears on the living egg as a small white spot; examined under a low magnifying power it shows a distinct radial structure. This polar figure is the outward expression of the radial lines around the external pole of the amphiaster. About twenty minutes after deposit there appears exactly in the centre of this figure a *pellucid spot.* This spot is free from deutoplasmic elements, but appears dark in consequence of the opaque background. This spot is the centre of the outer pole of the amphiaster. The polar figure not only reveals the position of the amphiaster, but also determines the main axis of the egg and at the same time the axis of the future embryo. It marks very nearly, at least, the place where the future mouth forms, and this pole of the egg may be therefore called the *oral pole*, and the opposite the *aboral pole.*

Polar Globules.— About thirty minutes after deposit an interesting change takes place. A well defined constriction appears in the equatorial plane of the egg, and passes by a sort of peristaltic movement toward that pole of the egg which bears the polar figure. In the course of ten minutes it passes off at this pole, leaving only a nipple-like protuberance. In a few minutes this protuberance is seen to be a transparent spherical body in process of liberation. Soon it is fully eliminated and lies between the yolk and the egg-membrane. Thirty minutes later a second body, like the first, is expelled, though accompanied by a much less clearly defined constriction. These two bodies are the so-called "*polar globules*," or "*directive corpuscles.*" They are of very general occurrence in the eggs of animals as well as the eggs of plants, but the constriction attending their exit above described, has not been observed in any egg save that of Clepsine.

Whence come these polar globules? If we make sections of the egg, at the time the constriction is passing, parallel to the axis of the egg, we shall, if successful, obtain one section which includes this axis. In this section we shall find the amphiaster in process of division, and we shall see that the polar globule represents the external half of the amphiaster, together with a little of the protoplasm of the egg. It contains then nuclear substance and cell-protoplasm, in other words, is a genuine cell. The remaining half of the amphiaster is soon transformed into a complete amphiaster, which divides, and produces the second polar globule. The half of the last amphiaster which still remains in the egg is not changed into a third amphiaster, but assumes the form of one of the amphiastral poles. This stellate figure is the *female pronucleus.* Near the opposite pole of the egg a second stellate figure is seen. This is the *male pronucleus.* It owes its origin to a spermatozoon which has previously entered the yolk.

Cleavage-nucleus.—In the female pronucleus two small bodies are seen in close apposition, and in the male pronucleus, one such body. These bodies are nucleoli. The two pronuclei begin to approach each other, as if by reciprocal attraction, meet near the centre of the egg and here blend into a single nucleus—the cleavage-nucleus, so called because it is the nucleus which introduces the cleavage. The three nucleoli also meet in the centre of the cleavage-nucleus, but do not blend until about the time the cleavage begins. From this it appears that *impregnation consists in the complete fusion of corresponding parts of two sexually differentiated cells—the egg-cell and the sperm-cell.*

Polarity.—While the phenomena thus far described—polar figure, pellucid spot, and polar globules—have been confined to one pole of the egg, those which are to follow are repeated on both poles of the egg. A short period of uni-polar activity is followed by a longer period of bi-polar activity. In the latter period the pole thus far active, still asserts its preëminence by taking the lead in actions that repeat themselves later and more sluggishly on the opposite pole.

Polar rings.—I have now to describe a phenomenon thus far known only in the egg of Clepsine. About one hour and thirty minutes after deposit, a shallow groove forms around the oral pole, and in this groove a transparent fluid collects, thus forming a conspicuous polar ring. Soon the equatorial side of this ring becomes denticulate or rayed. Ten minutes later a similar ring forms around the aboral pole, with rays from its equatorial side. The life of the egg at this time seems to be concentrated in this outward display of polarity. These rings begin to advance slowly, each toward the pole it incloses; meanwhile the rays have reached their maximum intensity and begin to dwindle. The oral ring advances in such a manner that it forms a constriction about the inclosed yolk, forming of this a kind of flat cap, or calotte.

The aboral ring does not strike deep enough to form a calotte, but seems to pass over the pole, forcing the inclosed yolk in towards the centre of the egg. This concentration of the rings continues until the aboral ring has the form of a disc, and the oral ring is pierced only by a slender column of yolk, on which rests the calotte. If we make a median section of the egg at this time, the aboral disc will present an oblong elliptical form, while the oral ring will appear as two separate parts, divided by the slender column of yolk which supports the calotte. Each of these parts has a sharply outlined cordate form. At this time the nucleus will be found a little eccentric, lying nearer the oral than the aboral pole. The three nucleoli have maintained their identity and relative positions thus far. A few minutes later the ring-substance loses its sharp outlines and begins to flow slowly towards the centre of the egg. At this moment the three nucleoli become invisible, and the nucleus assumes the amphiastral form already described. This amphiaster is much larger than those before mentioned, and its division will result, not in the production of a diminutive cell, or polar globule, but in the sundering of the egg into two nearly equal parts. It is interesting to note the behavior of the two poles of the amphiaster while the egg is dividing. The cleavage-groove begins first on the oral side of the egg — on that side nearest to which the nucleus lies. At the moment it begins we find that the two amphiastral poles are much farther apart than at first, and that the radial polar lines are much more intense. As the cleavage advances the two poles move still farther apart and the radial lines reach their maximum intensity. It is plain to see that the power which cleaves the egg resides in the nucleus. During the cleavage the two poles of the amphiaster act as if repelling each other, moving farther and farther apart and pulling the passive yolk elements with them. Thus they appear as centres of attraction for the yolk.

Approach of the two cleavage spheres.— As soon as the cleavage is completed, the two parts, instead of parting company, begin to approach, flattening against each other, until at length they together form a perfect sphere — as perfect as the undivided egg. The line of division is not, however, obliterated. Why do the two parts of the egg approach in this manner? This fact has been often noted and has been generally explained as the result of pressure exerted by the elastic membrane of the egg. This explanation, however, cannot apply to those cases where the egg lies at a great distance from its membrane, as in the case of many Molluscan eggs. I have good evidence that it does not apply in any case. I once liberated an egg from its membrane, and thus had an opportunity to follow the cleavage unhindered by the membrane. At the close of the cleavage the two parts touched each other only at a common point. Subsequently they began to approach, flattening on their contiguous faces, and finally formed together a perfect sphere. If we follow the two nuclei that have resulted from the division of the amphiaster, during the approach, we find that they take the lead in the movement. The cleavage as well as the subsequent approach of the cleavage-spheres are therefore due to nuclear influence.

"*Faltenkranz.*"—In conclusion I will call attention to some interesting radial phenomena which have never been satisfactorily explained.

Kleinenberg, in his well known work on the development of Hydra, states that the cleavage begins on one side of the egg, and that pseudopodial processes arise from the walls of the cleavage-groove, on this side. Metschnikoff observed folds on the walls of the cleavage-groove in the eggs of certain Siphonophores. As long ago as 1824 similar folds ("Faltenkranz," Reichert) were observed by Prévost and Dumas on the Amphibian egg. These folds were seen by von Baer, and more recently by Götte. They have been made a subject of special study by Max Schultze and Reichert. According to every explanation thus far offered of these folds, they should appear at right angles to the plane of cleavage in its entire circuit. This, however, is not the case. They appear only on one side of the egg, and instead of being at right angles to the plane of cleavage, they are at right angles only at the centre, diverging more and more towards either end of the groove, thus forming a kind of long star. From what has before been said, it is evident that the radial influence of the two poles of the amphiaster is greatest at the moment the cleavage appears, and that the cleavage begins on one side, rather than on all sides simultaneously, because the nucleus lies eccentrically—always nearer the side on which the cleavage begins. It seems, therefore, highly probable that these "processes" and "folds" are merely the outward expression of the radial influence of the nucleus.[1]

[1] For a more detailed account of the above phenomena, see Quart. Journ, Mic. Sci., July, 1878.

[Printed at the SALEM PRESS, February, 1879.]