A CONTRIBUTION TO OUR KNOWLEDGE OF THE DEVELOPMENT OF THE OYSTER (OSTREA EDULIS L.).

By DR. R. HORST.[*]

During the past summer, as our zoological station was then established in the vicinity of the oyster banks in the Eastern Schelde, I busied myself for several weeks with the study of the history of the development of the oyster. Even though this investigation is still incomplete, I think, however, that the following communication may contribute to an increase of our still very fragmentary knowledge of the embryology of the bivalve mollusca. These investigations were carried on in the station at Wemeldinge,[†] where, during my stay, I experienced many disinterested and important favors at the hands of MM. Zocher and De Leeuw. The study of the history of the development of the oyster is beset with peculiar difficulties, to which the French zoölogist, M. Lacaze-Duthiers, alludes as follows: "The oyster is certainly one of the most difficult of the species of the group of acephalous lamellibranchs to study, both in relation to its organization as well as its development."[‡] While in the case of most of the lower animals the sexes are confined to distinct individuals, and the sexual products, when mature, freely escape from the body, the fertilization taking place outside the latter, with the oyster this is not the case. Not only do the embryos pass through their first stages of development within the mantle cavity of the adult, and impregnation occurs internally instead of externally, but it may also be said that the eggs and spermatozoa come into contact in their passage out of the generative glands. If it is desired to observe the first changes of the fertilized egg, it is therefore impossible to resort to artificial impregnation as in the case of most other lower animals, and one is obliged to trust to finding individuals which are full of brood, which may be opened for the purpose. If a mother oyster is opened in the usual way, that is, by cutting through the adductor muscle, the animal soon dies, and the normal development of the brood which it contains is also disturbed; for one may keep the embryos alive in an aquarium for several days, though abnormal conditions soon make their appearance, if the development itself does not come to a complete standstill. Lacaze-Duthiers observes that he kept oyster larvæ alive in aquaria longer than a month, but he affirms that during all of this time slow changes of organization occurred, which it is safe to say were not nor-

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[*] Bijdrage tot de Kennis van de Ontwikkelingsgeschiedenis van de Oester (Ostrea edulis L.), door Dr. R. Horst, in Utrecht. Extracted from *Tijdschr. d. Ned. Dierk. Vereen*, dl. vi, 1882. Translated by J. A. RYDER. Abstr. in Zoolog. Anzeiger, 3d April, 1882.

[†] See the 6th yearly report (Jaarverslag) of the zool. station.)

[‡] Mém. sur le développement des acéphales lamellibranches. (Comptes rendu, cad. Sc., Paris, t. xxxix, p. 1197.)

mal. In one instance I was successful in making an opening at the edge of the shell of an adult, by which means the animal was very slightly, if at all, injured, and which enabled me to introduce a pipette into the mantle cavity in order to obtain embryos and to follow the undisturbed development for a couple of days; but this method was not long available, since every time embryos were detached artificially great numbers would escape from the parent, so that all of the brood was soon lost. It is, therefore, impossible to obtain an unbroken series of the different stages of development, but it is necessary to resort to the method of comparison of the observed stages, and in that way endeavor to form an idea of the mode of development. It is also a fact that one cannot always distinguish by external marks those adults which contain brood; the relaxation of the adductor muscle and the less energetic closure of the shell consequent upon that condition is a pretty sure indication that the oyster is full of embryos, but this does not remove our doubts as to the age of the brood and how soon it will be set free. I also obtained many more mother oysters containing old than young brood, and I would state here that in consequence of this fact the first stages of segmentation are in great part unknown to me, a hiatus which I hope to have the opportunity to fill up next season.

Davaine* has figured several of the first stages of the segmentation of the egg of *Ostrea edulis;* if these are compared with the stages observed by me, as in Figs. 1 and 2, and with those observed by Brooks† in his account of the development of *Ostrea virginiana*, there remains little doubt that the first stages of segmentation of the egg of the oyster take place in a manner similar to that of the eggs of other lamellibranchs. From the beginning of development onwards there is already a decided difference between the lowermost (vegetative) and the uppermost (animal) portion of the egg, so that after repeated cleavage the lowermost pole consists of a large granular cell, from which the entoderm (and mesoderm?) develop, while at the upper pole lie numerous smaller and clearer cells, which enter into the formation of the ectoderm (Fig. 1). These animal or ectoderm cells multiply by repeated fission and grow down over the vegetative pole more and more, until they finally close over and include it. Now the large entoderm cell or sphere also begins to divide, at first into two great round cells (Fig. 2), later into a number of cylindrical cells (Fig. 4); at the same time the embryo loses its spherical form, and after an invagination of the entoderm of the lower pole it assumes a slightly reniform shape, as seen from the side in Fig. 3, in which the uppermost pole is represented as directed upwards. If an older stage is now observed in longitudinal section (Fig. 4), it is seen that the entoderm cells are slightly invag-

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* Recherches sur la génération des Huîtres, Pl. II.

† The development of the American oyster (*Ostrea virginiana* List.). Studies from the Biological Laboratory of the Johns Hopkins University, No. IV, 1880, Pls. 1, 2, and 3.

inated, and that in this way a true gastrula stage has been developed. It is plain, however, that no true gastrulation takes place, since it is not possible to demonstrate a true cleavage cavity; indeed it appears to be, as it were, a transition form between a gastrula formed by embolic invagination and one developed by the epibolic downgrowth of the ectoderm over the entoderm. This last form appears to be common to other marine lamellibranchs. Indeed Rabl* has already pointed out that these apparently and fundamentally different modes of the formation of the gastrula are connected together by a series of transition forms, and that both may be referred to essentially the same process.

The embryo oyster at this stage is remarkable in that there is not only an invagination at the vegetative pole, but that there is also visible a distinct transverse groove formed a little below the apex of the opposite pole. When the embryo is viewed from the side the latter invagination immediately becomes apparent (Fig. 5, *sk*), and an optic section (Fig. 4) teaches us that it has originated from a mass of ectoderm cells which have been pushed inwards towards the center of the embryo. In the course of further development (Figs. 7 and 8), a sack with a narrow cavity is developed from this invagination, the walls of which are formed of long, cylindrical cells; the blind end of the sack is now directed towards the dorsal side of the embryo, whilst the direction of its cavity is parallel with the longitudinal axis of the latter. Without doubt, as we see in the case of older stages, this sack or invagination is nothing more than the shell gland. The assertion of Fol† that the shell gland in the embryos of *Ostrea* is not a true invagination, but that it is merely an ectodermal thickening, slightly hollowed out, is thus seen not to be very just, and apparently rests on what is observed in the older stages, where, as in other embryo mollusks provided with an external shell, the invagination becomes gradually shallower. As is well known, this organ was first observed in the *Cephalophora*, and was afterwards met with by Ray Lankester‡ and Hatschek§ amongst the lamellibranchs (*Pisidium* and *Teredo*); in comparison with the genera just named, the shell gland of the oyster appears very early in embryonic life.

The first investigators who studied the development of the oyster, Davaine and Lacaze-Duthiers, speak of "une échancrure" and "une dépression" from the presence of which the embryo becomes heart-shaped when viewed from the side; this invagination therefore appears to have been known to the older authors, although its significance was not understood by them. According to the investigations of Brooks, the embryo of *Ostrea virginiana* also has a deep depression or groove on

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* Entwickelung der Tellerschnecke. Morph. Jahrbuch, Bd. V, p. 601.

† Études sur le développement des Mollusques. Arch. de Zoologie ixpér., T. vi, p. 186.

‡ On the developmental history of Mollusca. Philos. Transac. Roy. Soc., 1874.

§ Ueber Entwickelungsgeschichte von Teredo. Arbeiten aus dem Zool. Inst. Wien., T. III.

dorsal side, which he considers the external opening of the gastrula—the blastopore. If, however, we compare his Fig. 32 (*op. cit.*) with my figures 5, 6, and 8, then I believe that we may infer with great probability that the structure regarded as the blastopore by Brooks is nothing more than the external opening of the shell gland. This view is further sustained by the fact that he observed that at a later period the shell began to develop at this point, regarded by him as the opening of the blastopore. Such a mode of development of the shell of lamellibranchs has hitherto been observed only by Rabl* in *Unio*, and is so entirely opposed to the observations which have been made on the development of other lamellibranchs that, as has been capably observed, the matter should be more closely reinvestigated.

Returning to the embryo represented in Fig. 6, we see that the entodermal field or area, which in an earlier stage (Fig. 4) presents as yet little more than a slight depression, has now acquired the form of a deep invagination with a tubular cavity, the true gastrula form (protogaster); behind the mouth of the gastrular opening lie a pair of large cells, which may apparently be regarded as the first mesodermal cells, although their mode of origin as well as their further development I have failed to discover. In the embryo of the following day (Fig. 8) one already encounters mesoderm cells on the dorsal side of the rudimentary intestine. The ventral portion of the embryo which lies below the mouth now begins to be pushed out, so that a foot-like prominence is developed, whereby the embryo assumes some likeness to a young gastropod. The blastopore is still very distinctly visible, and has a peculiar triangular form, as seen from the anterior end, as in Fig. 7. As far as I have been able to make out, the blastopore does not close, but is transformed directly into the permanent mouth. For even in those forms in which the blastopore closes, the œsophagus as well as the permanent mouth is formed by an invagination of the ectoderm and also in those in which the blastopore does not close, the ectoderm cells have a share in building up the anterior portion of the alimentary canal.

During the further growth of the embryo, great internal as well as external changes take place; which is true in the first place in regard to the shell gland, which gradually loses its original character of a glandular invagination; its walls are reflected outwards, so that it again becomes merely a shallow depression, with a thickened floor of long conical cells (Fig. 9, *sk*). A cuticular membrane, *s*, the product of the secretion of these cells, represents the primitive foundation of the shell, and upon this point, in the full grown animal, rests the hinge. Accordingly, the representations of Davaine, who remarks, "Un trait transparent * * * * c'est le premier indice de la charnière," are fully borne out. The bivalve shell of the oyster is thus plainly seen to develop from

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* Ueber die Entwickelungsgeschichte der Malermuschel. (Jen. Zeitschrift, XI., 1876.)

a simple unpaired rudiment, in opposition to the observations of Lacaze-Duthiers, according to whom both halves of the shell originate, "par deux boursouflements de l'enveloppe", (?) which afterwards unite to form the hinge. Brooks, in discussing this point recently in regard to the American oyster, observes that the shell from the first consists of two distinct halves, which develop from a small, irregular, transparent tract which lies in and athwart the dorsal groove or depression—his blastopore.

If it is also borne in mind, as I have before observed, that the peculiar character of this groove and the true blastopore have apparently escaped the observation of the last-named author, then we may be justly allowed to entertain some doubts as to the correctness of his interpretation. On the other hand, the description given by Hatschek of the first appearance of the shell in *Teredo*, agrees perfectly with that observed by me in *Ostrea*, and we may, as it appears to me, with safety assume that *the development of the shell in all mollusks takes place in the same way.* This admits of no question; and, as the last-named investigator very justly observes, it is a weighty argument in support of the position so ably defended by Von Jhering, viz, the theory of the monophyletic descent of the Mollusca.

Meanwhile, the ectoderm frees itself over almost the whole circumference of the embryo from the entoderm, so that now, for the first time, a body cavity (segmentation cavity) becomes apparent; a crown of cilia is also developed above the mouth, and the velar area (included by the ciliary girdle) is composed of columnar cells (Fig. 9). The entoderm has, meanwhile, enlarged and includes a spacious stomach cavity, from which a sac-like outgrowth is developed below, which still ends blindly, but which will afterwards be fused with the ectoderm to form the anal end of the intestine.

In the stage of development attained by the next day (Fig. 10), the shell has grown considerably in size. It now covers a large portion of the body, and, as indicated by treatment with acids, is already in part composed of lime carbonate. After the application of dilute acetic acid there remained only a tough membrane of conchioline. The ectoderm cells, which lie below the shell, have by this time become extremely flattened and transparent, so that one can no longer make out their contours, and with difficulty their highly refringent nuclei. The larva (Fig. 11), which now takes in nourishment, moves about with a lively motion and begins to grow slowly; the velum now forms a prominent portion of the body, which will be entirely covered by the shell as the latter grows larger. The velar area, which is included by the ciliary crown of the velum, is already slightly thickened in the center, the rudiment of the velar plate. A funnel-shaped œsophagus passes into the wide pear-shaped stomach which communicates posteriorly with the exterior through the intermediation of the intestine.

After the appearance of pigment on various parts of the body (velar plate, œsophagus, and blind saccular portion of the stomach), the brood begins to assume a gray or bluish color. The dimensions of the valves are now $0^{mm}.16$ (about $\frac{1}{160}$ of an inch); their form is almost circular, except the hinge border, which is straight. As already noted by Lacaze-Duthiers, the hinge at this stage is already provided with teeth. One may now note that the whole animal is withdrawn within the shell from time to time. This is effected principally by the help of a dorsal and a ventral muscle, *ds* and *vs*, which originate near the hinge border, and are inserted at the base of the velum. These muscles are formed of branched, attenuated, mesodermal cells, the branches of which traverse the body cavity in various directions. Several of these cells, which extend across from the left to the right half of the shell, have been aggregated into a group, and form a distinct adductor muscle, *sp*. Whenever the larva swims it thrusts the head or velar end of the body out of the shell, and partly turns it outwards over the edges of the latter anteriorly. The preoral ciliary crown consists of a double row of long cilia. If the velar area is viewed from above, the cilia will be found implanted upon two nearly approximated rows of almost rectangular cells. From each of these cells two cilia arise, which in stained preparations may be traced for some distance into the cellular protoplasm. I was unable to detect a postoral ciliary band, although the cephalic extremity of the embryo behind the preoral band is clothed with cilia. The velar area now consists in great part of a single layer of very much flattened cells, which can scarcely be defined even when aided by the presence of their stained nuclei; only in the center is there a marked thickening, which projects inwards, composed of distinct layers of ectodermal cells (*tp*). This is the structure which we have heretofore been designating by the name of velar area (*topplaat;* German, *Scheitelplatte*), and from which the supraœsophageal ganglion is developed. A longitudinal groove appears to divide this area superficially into two halves, but in consequence of a black pigment which is usually developed in this region, I could not make it out distinctly. Peripheral nerves, which pass outwards from the central velar thickening, such as were observed in the larvæ of *Teredo* by Hatschek, were not encountered by me. The above-mentioned ectodermal thickening appears to have been noticed in the larval oyster by Davaine, as well as by Lacaze-Duthiers, but was at first regarded by both as the oral opening—an error which was afterwards rectified by the last-named investigator.

Together with the other parts of the body the intestinal canal has also progressed in development, the œsophagus, which has been pigmented with a brown color, has grown longer, and its anterior portion has been widened, and become funnel-shaped. The cavity of the stomach has grown in length, and a constriction divides it into an upper and a lower portion. From the upper portion on the left and right sides a large round blind sac (*l*) has been developed, which constitutes the rudiment of the liver, while at the level of the constriction the intestine arises, making a couple of bends upon itself before opening into the mantle cavity (*mh*). The entire internal surface of the alimentary canal is clothed with cilia, with apparently the exception of the hepatic diverticulum (*l*), the internal surface of which it is difficult to observe in consequence of the presence of a black pigment.

At the point where the rudimentary foot was formerly developed, a thickening of the ectoderm is now formed, of the same character as that already described as arising in the center of the velar area. This mass also contains a large number of nuclei; but whether the pedal ganglion is developed from it, I have not been able to make out, nor could I discover the presence of auditory vesicles, though Lacaze-Duthiers claims to have seen them. Nor was I more fortunate in detecting the presence of excretory organs, although I made special efforts to discover them; otherwise the numerous points of agreement, of the larva (trochophora) of the oyster with those presented by *Teredo*, were complete, with only these slight exceptions. Perhaps renewed investigation would show that the segmental organs also are not wanting. Older stages than that represented in Fig. 12 I was unfortunately not able to investigate, so that regarding the length of the period which intervenes between the time when the larvæ are set free, and the time at which they fix themselves, as well as the changes which they undergo during this period, I am unable to affirm anything.

The difficulties encountered in distinguishing the young oyster, immediately after it has attached itself, I believe I have entirely overcome: and instead of using the ordinary collector for this purpose, which is covered with a mixture of lime and sand, a collector should be used which is covered with an even coat of clean lime; for the numerous little asperities due to the presence of the sand grains; make it difficult even for the sharp-sighted oyster fishermen to distinguish the shells of the young oysters on the collectors of the usual form. In order to have the surface as even as possible, I used panes of glass instead of the usual form of collector, though this is not positively necessary. After one of these panes of glass had been immersed in the water for eight days, several young oysters were found to be attached; of these the largest measured $0^{mm}.85$ (about $\frac{1}{30}$ inch), and the smallest scarcely $0^{mm}.57$ (about $\frac{1}{43}$ inch) in diameter, though the latter was already visible to the naked eye.

The experiments instituted by Dr. De Leeuw and myself, in order to learn if the young oysters would fix themselves in inclosed basins, have not yet been completed.

Before closing, I would call attention in this place to a probable enemy of oyster brood. In my aquarium, in which a mother oyster was placed, and which every now and then threw out a great quantity of brood, there were also a couple of actiniæ of the same species which is very often observed attached to oyster shells. It occurred to me that the quantity of brood was diminishing too rapidly, and upon making an investigation of the matter I found a number of small bluish-gray pellets, a couple of millimeters in diameter, floating about in the water. Just then I saw one of the actiniæ eject a similar pellet from its mouth. Upon investigating one of these pellets microscopically I learned that it consisted of nothing else than the empty shells of young oysters, the remains of the ingested food of the actinia. Although I do not believe that the actiniæ, under ordinary natural conditions, have the opportunity to destroy as many larvæ as noted above, they may, however, be able to destroy great quantities, as they are present in great numbers, attached to the old oysters at the sea bottom.

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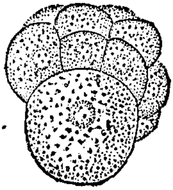

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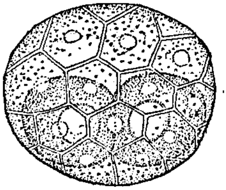

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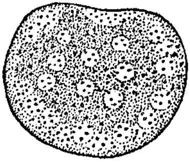

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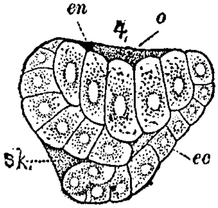

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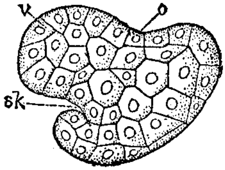

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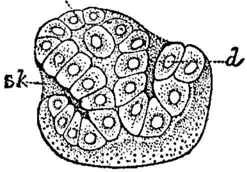

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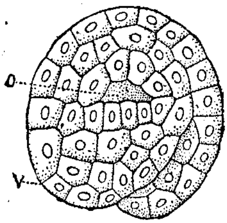

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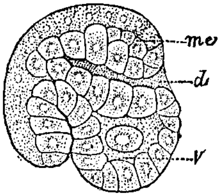

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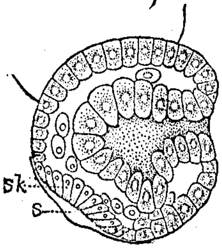

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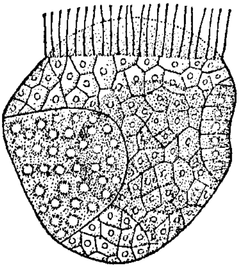

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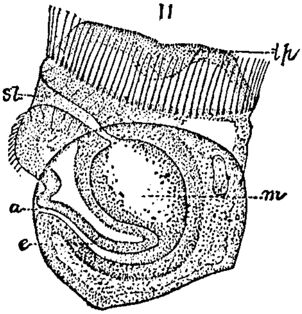

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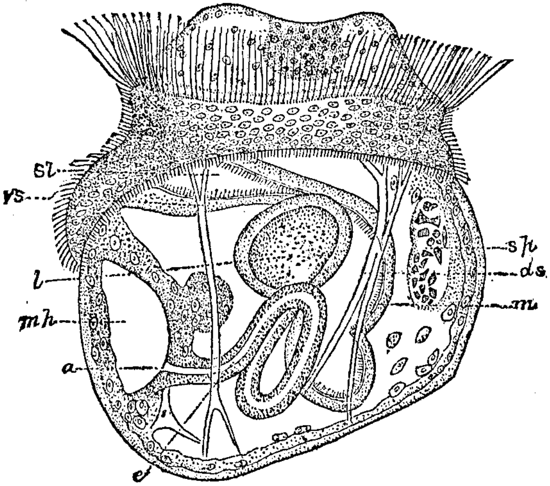

Explanation of the Plate.

FIG. 1.—Segmented egg showing the large vegetative cell below, and the numerous animal cells above.

FIG. 2.—Older stage, seen from above, showing the two entodermal cells shimmering through from below.

FIG. 3.—Embryo seen from the side, showing the commencement of the gastrular invagination.

FIG. 4.—More advanced stage, seen in optic section, showing the entodermal invagination and the commencement of the shell gland; *ec*, ectoderm; *en*, entoderm; *o*, gastrula mouth—blastopore; *sk*, shell gland.

FIG. 5.—Still older embryo, seen from the side; *v*, foot; the other letters with the same signification as before.

FIG. 6.—The same stage seen in optic section; *me*, mesoderm; *d*, gastrula cavity or archenteron.

FIG. 7.—Embryo one day older, seen from the anterior end, showing the triangular opening of the blastopore.

FIG. 8.—The same stage in optic section.

FIG. 9.—Embryo one day older, seen in section, with ciliary crown (vellum), stomach cavity, and rudiment of the shell developed; *s*, shell.

FIG. 10.—A more advanced stage seen from the side, with the shell further developed.

FIG. 11.—Larva still more advanced, with velar disk or area developing, the central thickening of which, *tp*, is apparent; *a*, anus; *e*, rectal end of intestine; *m*, stomach; *sl*, œsophagus; *tp*, rudiment of supraœsophageal ganglion.

FIG. 12.—More advanced larva with a double preoral girdle of cilia, hepatic sac, muscles, and rudiment of supraœsophageal ganglion developed; *ds*, dorsal pallial muscle; *vs*, ventral pallial muscle; *sp*, adductor muscle; *l*, hepatic sac or diverticulum of the stomach; *mh*, mantle cavity. The remaining letters have the same signification as in previous figures.