Lillie

PRELIMINARY ACCOUNT OF THE EMBRYOLOGY OF UNIO COMPLANATA.

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THE following paper is preliminary to a more extended one, which I hope to publish soon. In the complete paper I shall advance the proof of the statements made here.

In carrying out the work two main objects have been kept in mind:—

Firstly to settle definitely the question of the origin of the germ layers in the Unionidae, and

Secondly to extend the cytogenetic method in embryological research to the class of the Lamellibranchs; a thing which has not hitherto been attempted in a very minute fashion. In the attempt to attain to these two main objects of the work, other problems of greater or less interest have presented themselves.

< 80.
× 70.
3. × 50.

Rabl[1] made a fundamental error in the interpretation of the shell-gland as the invagination of the entoderm. Balfour, while provisionally accepting Rabl's account, nevertheless calls attention in his text-book to the strong inherent improbability of the dorsal position of the blastopore. In 1891 Goette[2] published an account of his observations on the formation of the entoderm in Anodonta, and figured its invagination at the very region to which Rabl described it as wandering, after its supposed formation dorsally. In Unio the invagination takes place in the same region as described for Anodonta by Goette, but in an earlier stage of development.

Although Goette cleared up to a certain extent the question of the origin of the entoderm, he did not do so for the mesoderm; and for want of any contradiction, based on observation of the facts, Rabl's account of the origin of the mesoderm

[1] Rabl, Ueber Entw. der Malermuschel. Jen. Zeitschr. f. Naturwiss. Bd. X. pp. 310–393.

[2] Goette, Bemerkungen über die embr. Entw. von A. piscinalis. Zeitschr. f. wiss. Zool. lii.

teloblasts from the cells of the shell-gland, remains as the status of our present knowledge on the subject. I shall show that the origin of the mesoderm teloblasts in Unio is exactly the same as in Nereis, according to Wilson's[1] description. In addition to this yet another source of origin exists in Unio, *viz.*, from a cell on the left side of the young embryo of forty-six cells (Fig. 4, *Y*). I shall call the mesoblast derived from this source the *larval mesoblast*, inasmuch as it gives rise to structures which are *purely larval*.

SEGMENTATION.

The first plane of division is inclined at an angle of 45° to the future sagittal and transverse axes of the larva. It runs from the animal to the vegetative pole, and divides the egg into two unequal parts, of which the smaller *A B* is anterior to the larger *C D*. Both cells contain entoderm as well as ectoderm, and, therefore, Rabl's designation of animal and vegetative does not correspond with fact. The second plane of division runs likewise from the animal to the vegetative pole, and is practically at right angles to the first. It does not divide both cells at the same time. *C D* is the first to divide ; the two resulting cells are of unequal size, and the smaller *C* lies on the right side of the future embryo, while *D* occupies the median posterior region (Fig. 1). *A B* divides shortly afterwards and into parts approximately equal, one of which *A* (*slightly the larger*) lies on the left side and the other *B* occupies the median anterior region. The median longitudinal axis of the future embryo runs through the centres of *B* and *D* (Fig. 1 *m. l. p.*).

So far my description of the segmentation agrees completely with Rabl's and Flemming's[2]; but my interpretation of the value of these first four blastomeres, and their axial relations, is entirely different. Rabl held that the large posterior cell, *D* (1 of Rabl), was chiefly entodermic, while, as a matter of fact, it contains only a very minute portion of entoderm; that

[1] Wilson, Cell-Lineage of Nereis. Journal of Morphology. Vol. VI, No. 3.
[2] Flemming, Studien in der Entw. der Najaden. Sitzungber. der Wiener Akad. 1875.

the three other cells were purely ectodermic, while, in fact, all contain entoderm. Rabl was right so far that all four cells contain ectoderm, but so do all contain entoderm; while *D* contains the posterior mesoblast, and *A* the larval mesoblast.

The next stage to which I will direct special attention is the eight-cell stage (Fig. 2), which has the typical form of four apical micromeres lying on four macromeres, and alternating with them (being given off in a right-handed spiral). Here I must differ from Rabl's account. The four micromeres do not, indeed, arise simultaneously, but one after the other, and generally in the following order, $d,^1$ $c,^1$ $a,^1$ $b,^1$ from *D*, *C*, *A*, and *B*, respectively. The last three arise some time after $d,^1$ and very nearly together. These four cells form the first generation of micromeres, and are purely ectodermic. According to Rabl d^1 (5 of Rabl) divides at the same time as *C* (3 of Rabl). Whether the difference observed is due to the difference of species or not, I am unable to say.

The sixteen-cell stage does not actually occur as such, but the stage with seventeen cells corresponds to the sixteen-cell stage of Annelids, with one further division, which, in the Annelid ovum, occurs later. This stage does not arise at once from the eight-cell stage, but almost all the cells of the eight-cell stage divide at different times. The four macromeres are the first to divide, and of these *D* takes the lead, dividing by an equatorial plane into two unequal parts, of which the smaller *D* lies on the vegetative pole, and the larger d^2 between *D* and c^1 and d^1 of the apical pole. *C*, *A*, and *B* divide next in the same plane as *D*, and in this general order. In each case the smaller product lies on the vegetative pole. I have, therefore, reserved the term macromere, and the designation by capital letters for these cells. The three larger cells are $c,^2$ $a,^2$ and b^2; together with d^2 these cells form the second generation of micromeres. It is interesting to note that the second generation of micromeres is given off from the macromeres in a left-handed spiral; thus the reverse of the first generation; and that the third generation is given off in a right-handed spiral again (Figs. 3 and 4). The spiral arrange-

ment of the cells is caused not so much by a rotation of the cells when formed, as by the direction of the spindles during the division. The same thing has been noted by Wilson.

After the divisions of the macromeres are completed, a^1, b^1, c^1 and d^1 divide at different times, giving rise to eight apical cells, alternating with one another in the typical way. During the division of the apical cells a spindle forms in d^2 and a small cell is segmented off on the right side of the vegetative pole (x^1 Fig. 3).

I must remark here on the unsuitability, in this case and possibly in others, of the terms macromere and micromere. If we use the terms according to their etymological significance, a^2 b^2 c^2 and d^2 are the macromeres, and A, B, C, and D the micromeres; functionally the reverse is the case, inasmuch as A, B, C, and D, like the macromeres of Nereis, contain the entoderm.

The cell d^2 corresponds exactly to the cell d^2 or X of Wilson, which is the "first somatoblast." In the case of Nereis this cell forms the ventral plate and the mid-dorsal region as far as the prototroch.[2] In Unio it forms the shell-gland, and I think, that I can show, that in addition it forms the foot.

In general the apical pole lags far behind the vegetative pole in the further divisions, so that in the fifty-cell stage there are still only sixteen apical cells. This fact has made it impossible for me to follow the divisions of the apical pole cells to the late stage to which Wilson has followed them in Nereis, and Conklin[1] in Crepidula.

Let us now fix our attention upon the vegetative pole. Another division of A, B, C, and D takes place, giving rise in a right-handed spiral to the third generation of micromeres, a^3 b^3 c^3 and d^3 respectively. Shortly afterwards a fourth division of D takes place. This division is very unequal, the vegetative part D being much smaller than the part posterior

[1] Conklin, Preliminary Note on the Embryology of Crepidula fornicata and of Urosalpinx cinerea. Johns Hopkins Univ. Circulars, X, no. 88.

[2] This is true of the younger stages only; later the products of the first somatoblast become separated from the prototroch.

to it, *M* (Fig. 4). This cell lies between *X* and *D*, and corresponds exactly to the second somatoblast or mesoderm proteloblast of Nereis. Without further discussion I will simply say, that it is the proteloblast of the mesoderm in Unio, which is thus in every way identical with the mesoderm of Annelids (Wilson), and of Gasteropods (*vide* Conklin on Crepidula, and Erlanger[1] on Bithynia). Shortly afterwards *M* divides equally in a sagittal plane.

If so far the minute agreement of the segmentation with that of Nereis is surprising, I must confess that it seems little less than astounding that the next divisions should agree. To quote Wilson (Cell-lineage of Nereis, p. 411) : "At the second division each of the primary mesoblasts buds forth a small cell at the surface near its anterior margin." Exactly the same thing occurs in Unio, though I cannot follow Wilson further and say, "the budding of the mesoblasts is continued in the same way for a considerable period." As a matter of fact I have observed only this one superficial cleavage of the mesoblasts, which are shortly afterwards, at the time of gastrulation, taken within the segmentation cavity, where each forms a "band" of cells.

Let us return to the cell d^2 or *X*. Here again we notice an extremely close resemblance to the same cell in Nereis. There is, however, one difference. In Nereis *X* buds off three small cells before its division into two equal parts. In Unio four such cells arise from *X*. These four divisions are as follows :—x^1 segmented off on the right side of the vegetative pole, x^2 on the left side of the same pole, $x3$ in the median line towards the apical pole (and were a prototroch present in Unio, such as is present in Nereis, it would cause a similar median dorsal interruption of it), and x^4 in the median plane of the vegetative pole. (Not present in Nereis.) (Fig. 4.) *X* then divides sagittally and equally, and each half as in Nereis soon buds forth a small cell towards the vegetative pole. By continued division of the two large cells *Xl* and *Xr*, a plate of

[1] Erlanger, Beiträge zur Entw. der Gasteropoden. Mitth. aus der Zool. Stat. zu Neapel, Bd. X, Heft. 3.

large cells is produced dorsally, which invaginates *after gastrulation* to form the shell-gland (Figs. 5 and 6).[1]

I have already described the origin of a^2; it is from this cell that the larval mesoblast arises. As this is a point of some importance, and is open to doubt on *a priori* grounds, I shall describe the process in some detail. a^2 divides by an obliquely equatorial plane into $a^{2.1}$ and $a^{2.2}$ or *Y*. *Y* is larger than $a^{2.1}$ and lies nearer to the vegetative pole. *Y* next buds off a small cell y^1 to the right, which lies a little anterior to, and to the left of *M* (Fig. 4). Shortly after it buds off another small cell y^2 to the left. Again it divides and buds off y^3 posteriorly. *Y* is then gradually overgrown, and comes to lie in the segmentation cavity *anterior* to the entomeres. In the stage in which six large dorsal cells of the shell-gland are present, it divides sagittally into two equal parts. At about the time of the invagination of the entoderm each cell *Yl* and *Yr* divides into two parts, the dorsal product of which is in each case somewhat the larger (Fig. 5). But there is this distinction between the divisions of *M* and the divisions of *Y*: the divisions of *M* are typically teloblastic; those of *Y* are not (Figs. 5 and 6). The larval adductor muscle and some of the "Strangzellen" are formed from products of *Y*. These structures are purely larval, and take no part in the formation of adult tissues.

We have then in the formation and setting apart of this cell *Y* for this particular function an exceedingly instructive example of the precocious segregation of tissue elements. It seems as if in no other way could the adductor muscle, so important for the existence of the glochidium, be formed so early. Some light is thus thrown on the significance of the blastomeres of segmentation stages. It indicates that the mosaic arrangement is a derived condition, and has been acquired as the best means for the early separation of tissues.

The same cell in Nereis is the left stomatoblast (Wilson), which functions very differently. It may be interesting to

[1] It is interesting to note that in no other Mollusc has any cell been described comparable to the "first somatoblast" of Wilson. In the Gasteropods d^2 does not differ from the other micromeres of the same generation.

note that, according to Lang,[1] all of the second generation of micromeres in the Polyclad *Discocelis* contribute to the mesoblast, and that the larval mesoblast of Unio is part of one of the second generation.

Gastrulation.

I cannot state exactly how many cells enter into the formation of the entoderm, but whatever their number they are the products of *A*, *B*, *C*, and *D*. The third generation of micromeres is purely ectodermic. Gastrulation takes place at a stage when about twenty shell-gland cells are present. The invagination is never very deep, and in a superficial examination of whole embryos might easily escape observation altogether. That it has escaped observation hitherto is attributable to this fact. In section it is, however, very obvious (Fig. 5). Posterior to the blastopore in the segmentation cavity lie four cells, products of *M*, and anterior to it four cells, products of *Y* (Fig. 5). The blastopore occupies the posterior region of the body, and is afterwards occluded by products of *X* (Fig. 6). The anus arises later in the same area.

The oesophagus arises in the area described as oral plate ("Mundschild") by Flemming (Fig. 6, *oes.*). In some glochidia of Anodonta it is already in communication with the digestive tract, but as a rule the communication is not effected till post-embryonic stages, during the parasitic attachment of the glochidium to a fish.

Shell and Foot.

Interpreting the entoderm invagination of Rabl as shell-gland, I can only corroborate his account of the formation of the shell.

The cells which lie between the blastopore and the shell-gland are derivatives of *X* (Figs. 5 and 6). These cells grow past the blastopore in later stages and anterior to it as far as the mouth. When the whole ventral region of the

[1] Lang, Die Polycladen, p. 332. Monographie 11. Herausgegeben von der Zool. Stat. zu Neapel.

larva undergoes a longitudinal invagination to form the mantle, they form the bottom of the invagination as far forward as the mouth and become the anlage of the foot and pedal structures. The lateral pits are derived from the same source.

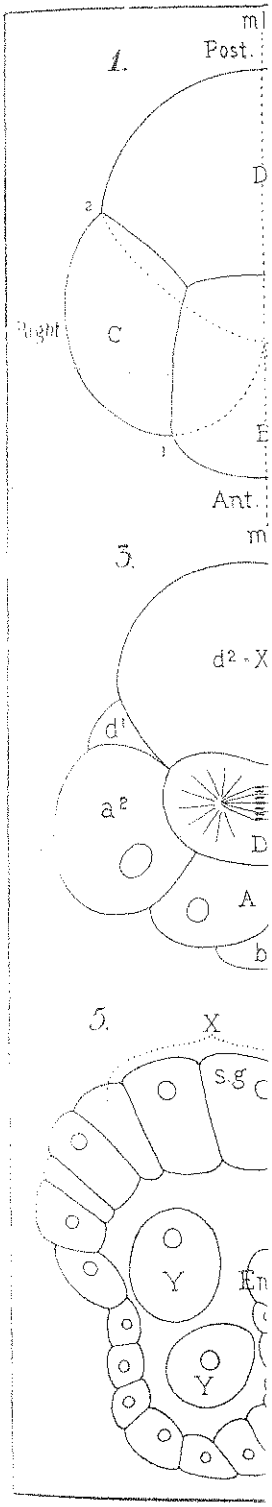
Thread-gland.

The larval byssus gland, for which I will adopt the better term, "thread-gland," proposed by Schierholz,[1] is a *unicellular* structure, which opens between five large cells placed just ventral to the anterior end of the shell-gland. Its duct is, therefore, *intracellular*. The cell from which it is derived is ectodermic, and in earlier stages lies in the centre of the five large cells just mentioned.

The complete paper will contain an account of the further development up to the glochidium stage.

UNIVERSITY OF CHICAGO,
Feb. 3, 1893.

[1] Schierholz, Ueber Entw. der Unioniden. Denkschr. der Math.-Naturwiss. Klasse Akad. Wien, LV. 1888.



EXPLANATION OF PLATE.

FIG. 1. Four-cell stage from the apical pole. 1–1 = First plane of segmentation. 2–2 = Second plane of segmentation. *m. l. p.* = Median longitudinal plane of future larva. *Post.* = posterior; *Ant.* = Anterior.

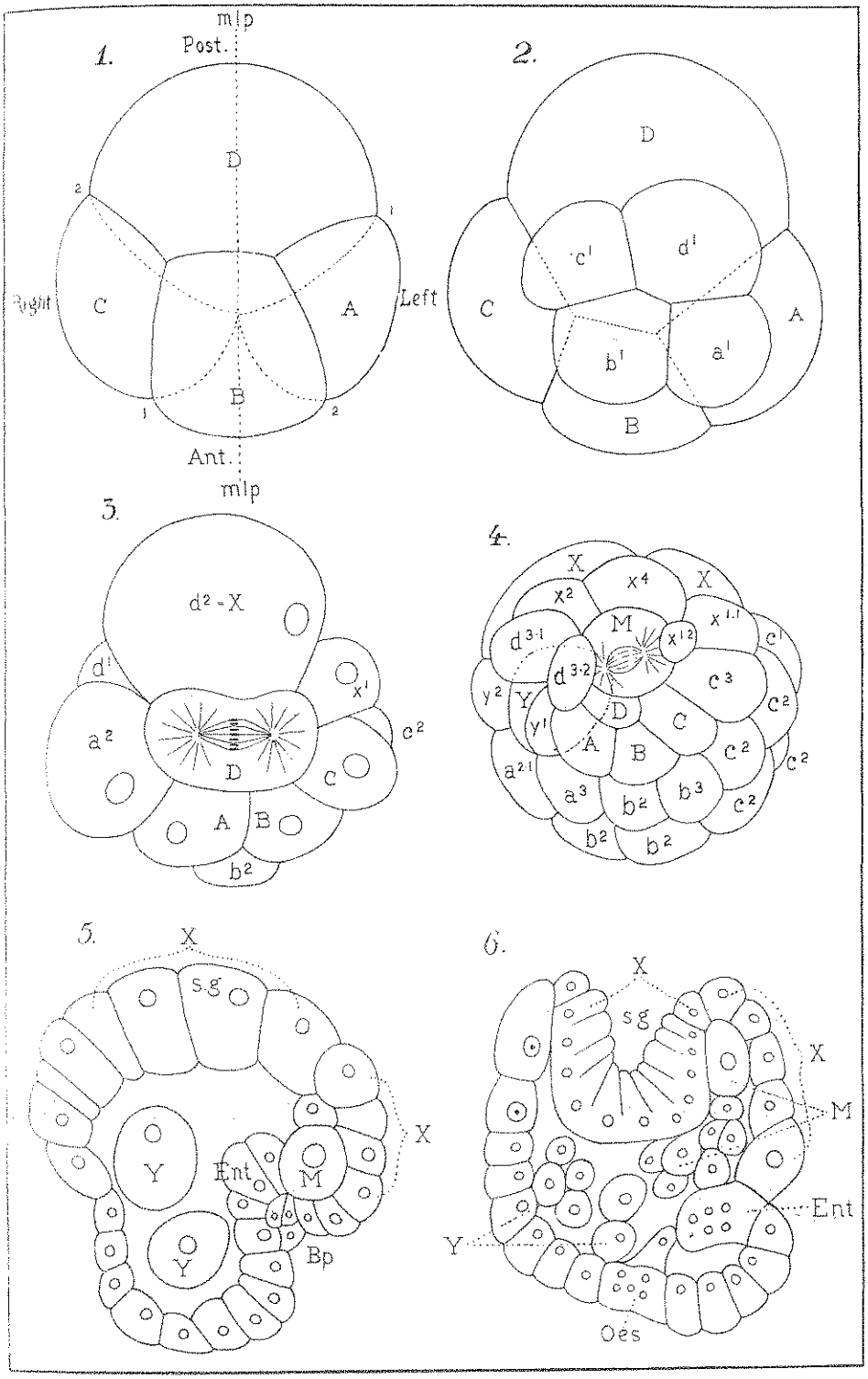
FIG. 2. Eight-cell stage from the apical pole. $a,^1$ $b,^1$ $c,^1$ d^1 the four apical micromeres derived from *A*, *B*, *C*, and *D*, respectively.

FIG. 3. Seventeen-cell stage from the vegetative pole. *A*, *B*, *C*, and *D* the four macromeres. $a,^2$ $b,^2$ $c,^2$ d^2 the second generation of micromeres. d^2 or *X* is the first somatoblast. x^1 is derived from *X* as indicated by the position of the nuclei. The spindle in *D* later separates d^3 to the left.

FIG. 4. Vegetative pole of forty-six-cell stage. *X*, $x,^{1.1}$ $x,^{1.2}$ $x,^2$ x^4 = products of first somatoblast *X*. *M* = second somatoblast or mesoderm proteloblast, derived from *D*. *Y* = larval mesoblast, derived from $a.^2$ y^1 and y^2 are products of *Y*. *A*, *B*, *C*, *D* = Entomeres.

FIG. 5. Sagittal section of young gastrula. *Bp.* = blastopore; *Ent.* = entoderm; *s. g.* = cells of shell-gland; *X* = products of the first somatoblast *X*; *M* = mesoderm teloblast of one side which has budded off one cell anteriorly. *Y* = larval mesoblast of one side.

FIG. 6. Sagittal section of an older stage. *Ent.* = entoderm; *s. g.* = shell-gland; *X*, *M*, and *Y* as above. *Oes.* = first indications of oesophageal invagination.


1.
m|p
Post.
D
C
A
B
Right
Left
Ant.
m|p
2.
D
C
A
B
3.
d² = X
d¹
a²
D
C
A
B
b²
4.
X
M
D
C
A
B
5.
X
s.g.
Y
Ent.
M
Bp
6.
X
sg
M
Ent
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Oes