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CONSIDERATIONS ON CELL-LINEAGE AND ANCESTRAL REMINISCENCE,

BASED ON

A RE-EXAMINATION OF SOME POINTS IN THE EARLY DEVELOPMENT OF ANNELIDS AND POLYCLADES.

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(Read December 13, 1897.)

FIVE years ago I observed in the embryos of two polychætous annelids, *Aricia fœtida* (Clap.) and *Spio fulginosus* (Clap.), that the two so-called "primary mesoblasts" bud forth a pair of extremely minute superficial cells near the posterior lip of the blastopore before giving rise to the mesoblast-bands.[1] Scarcely larger than polar bodies, these cells lie at or near the surface at the posterior margin of the entoblast-plate, wedged in between the latter and the primary mesoblasts (Fig. 1, *A*, *C*, *e*; Fig. 2, *A*, *e*, *e*); and in this position they are carried into the interior during the ensuing invagination. I could not determine their fate, and found no evidence that they underwent growth or division, or that they took any part in the building of the embryo. In *Nereis*, however, I found that this pair of rudimentary cells was represented by a group of not less than six or eight somewhat larger cells (Fig. 1, *B*, *D*; Fig. 2, *B*), formed in exactly the same way and in the same position,[2] and further that these

[1] 1892, p. 458. [2] 1892, p. 411.

cells were functional in development, giving rise to a definite part of the body, though, as will appear beyond, I fell into error regarding their precise fate.[1] These facts strongly suggested that the pair of rudimentary cells in *Aricia* and *Spio* were to be regarded as vestiges of an ancestral type of development in which they were represented by a group of larger functional cells, such as are still found in the embryo of *Nereis*. Such a conclusion, if it could be established, would possess an importance for the general problems of cell-lineage even greater than its interest for the more special problems of annelid embryology. For if vestigial structures may appear in ontogeny in the form of single cells, the fact would not only afford a striking illustration of the inadequacy of all so-called "mechanical" explanations of cleavage-forms, but would supply a very important datum for the estimation of the cell-theory as applied to development.

The results of a re-examination of the history of these small cells in *Nereis*, taken in connection with other recent studies in cell-lineage, lend strong support to the conclusion indicated above, enabling us, as I believe, to give a definite interpretation to the vestigial cells of *Aricia*, *Spio* and other forms in which they have recently been observed;[2] and they also raise some interesting further questions regarding ancestral reminiscence in cell-lineage. I am also able to contribute some new observations on the cell-lineage of a polyclade (*Leptoplana*), which bear directly on these questions and considerably extend their range.

[1] Von Wistinghausen (1891) had previously observed in *Nereis Dumerilii*, a group of small cells derived from the "second somatoblast," which probably correspond with those I have described in *N. limbata* and *N. megalops*, though their exact origin was not followed. Wistinghausen believed that they gave rise to a part of the ectoblast—a result wholly different from both my earlier account and the present one.

[2] Minute cells exactly corresponding in origin and number to those of *Aricia* have been found by Mead in *Amphitrite* (1894, p. 467; 1897, p. 247) and by Holmes in *Planorbis* (1897, p. 101). Lillie has found a pair of corresponding but slightly larger cells in *Unio* (1895, p. 27), while in *Clymenella* they are as large as the primary mesoblasts (Mead, 1897, p. 264). The corresponding cells in *Umbrella* (Heymons), *Crepidula* (Conklin), and *Physa* (Wierzejski) will be referred to beyond (see pp. 6, 11–12).

I.

The Relations between Mesoblast and Entoblast in Annelids and Mollusks.

In *Nereis*, as in the typical development of other annelids and of gasteropods and lamellibranchs, the mesoblast-bands are derived from the posterior cell of the fourth quartet of "micromeres."[1] This cell, now generally known as the *second somatoblast*, divides into two symmetrical halves which have been usually designated as the "primary mesoblasts;" and from them, by a series of slightly unequal successive divisions, arise the mesoblast-bands which extend forward in the cleavage-cavity at the sides of the embryo. Before giving rise to the mesoblast-bands, however, the "primary mesoblasts" bud forth the small cells already referred to, at or near the surface directly behind the two posterior macromeres "C" and "D." At least six, and probably not less than ten, of these cells are formed, the primary mesoblasts meanwhile sinking below the surface and becoming quite covered by ectoblast-cells which advance from the sides and from behind. The small cells first formed lie at the surface, wedged in between the "primary mesoblasts" and the macromeres (Fig. 1, *D*, *e*; Fig. 2, *B*,). Those formed later lie below the surface, owing to a change in the plane of division (Fig. 3, *A*). The small cells, which are very conspicuous in sections by reason of their intensely chromatic, closely reticulated nuclei, thus become arranged in a thin plate extending inwards from the surface between the primary mesoblasts and the two posterior macromeres (Fig. 3, *B*). After the formation of the small cells the divisions of the primary mesoblasts suddenly change both in form and direction, the plane of division being now nearly or quite at right angles to the former (*i. e.*, approximately parallel to the sagittal plane of the embryo) and the cells thus produced being nearly as large as the primary

[1] *Nereis* is somewhat exceptional in the fact that the other three cells of the fourth quartet are suppressed. In *Aricia*, *Polymnia*, *Spio*, *Pygmobranchus*, *Hydroides*, *Polygordius* (all of which I have examined), and in some others, the fourth quartet, is complete, and in the first two forms named, a fifth quartet of (entoblastic) micromeres is formed before the invagination (Cf. Fig. 2, *A*).

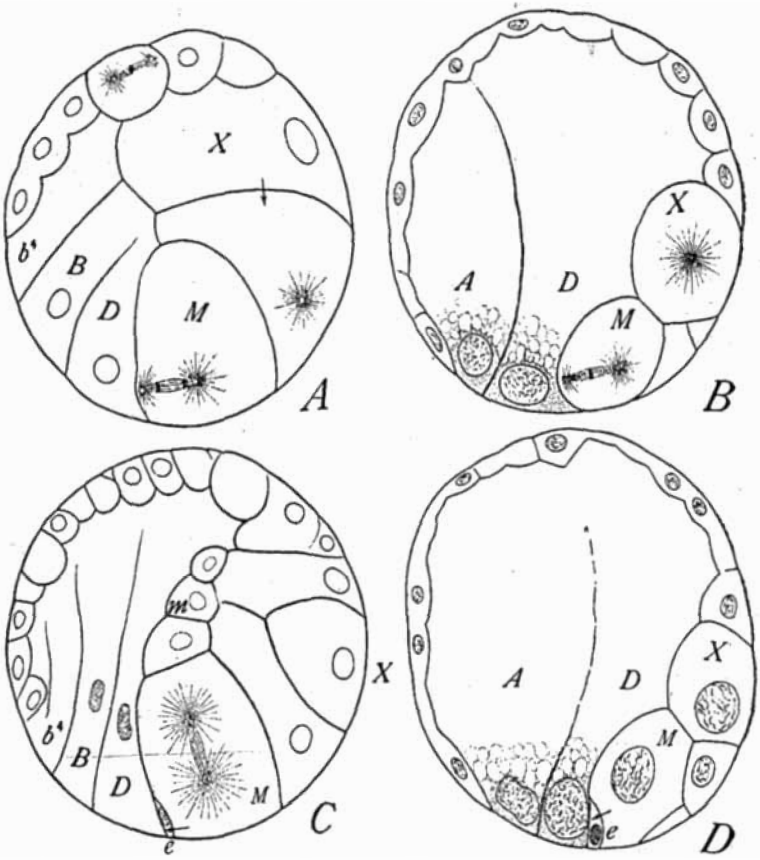
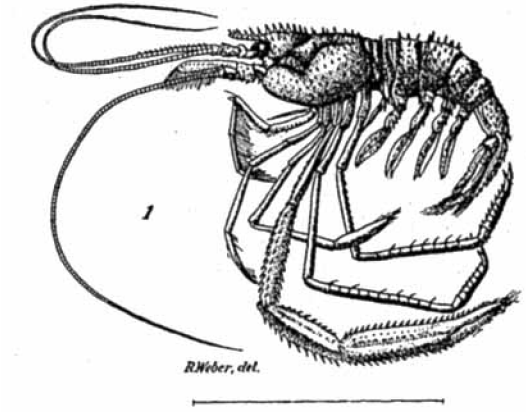


FIG. 1.[1] Early embryos of *Aricia* (**A**, **C**) and *Nereis* (**B**, **D**) in sagittal section (**A**, **B**, **C**, optical, **D**, actual). Showing the formation of small posterior entoblasts (*e*) between *M* and *D*.

A, *B*, *D*, *b*⁴, cells of the entoblast-plate (cf. Fig. 2); *M*, the "primary mesoblast;" *m*, mesoblast-band; *X*, the first somatoblast or its derivatives, forming the somatic plate.

mesoblasts. Thus are formed the mesoblast-bands which form together a V-shaped mass of cells lying between the macromeres and the overlying ectoblast. Near the middle line the two halves of the V are often slightly separated; and into the space

[1] All the figures are from camera drawings, made from preparations unless otherwise stated. Optical sections have been fully confirmed by actual.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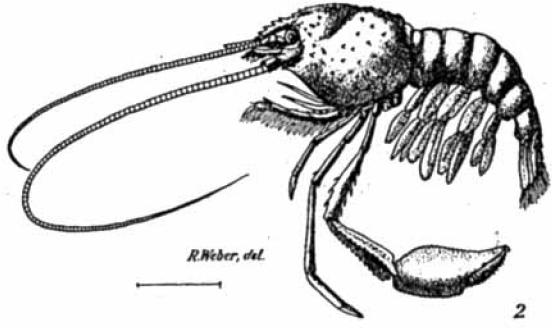
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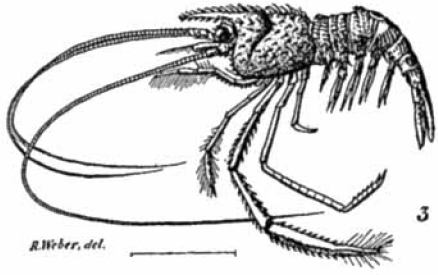
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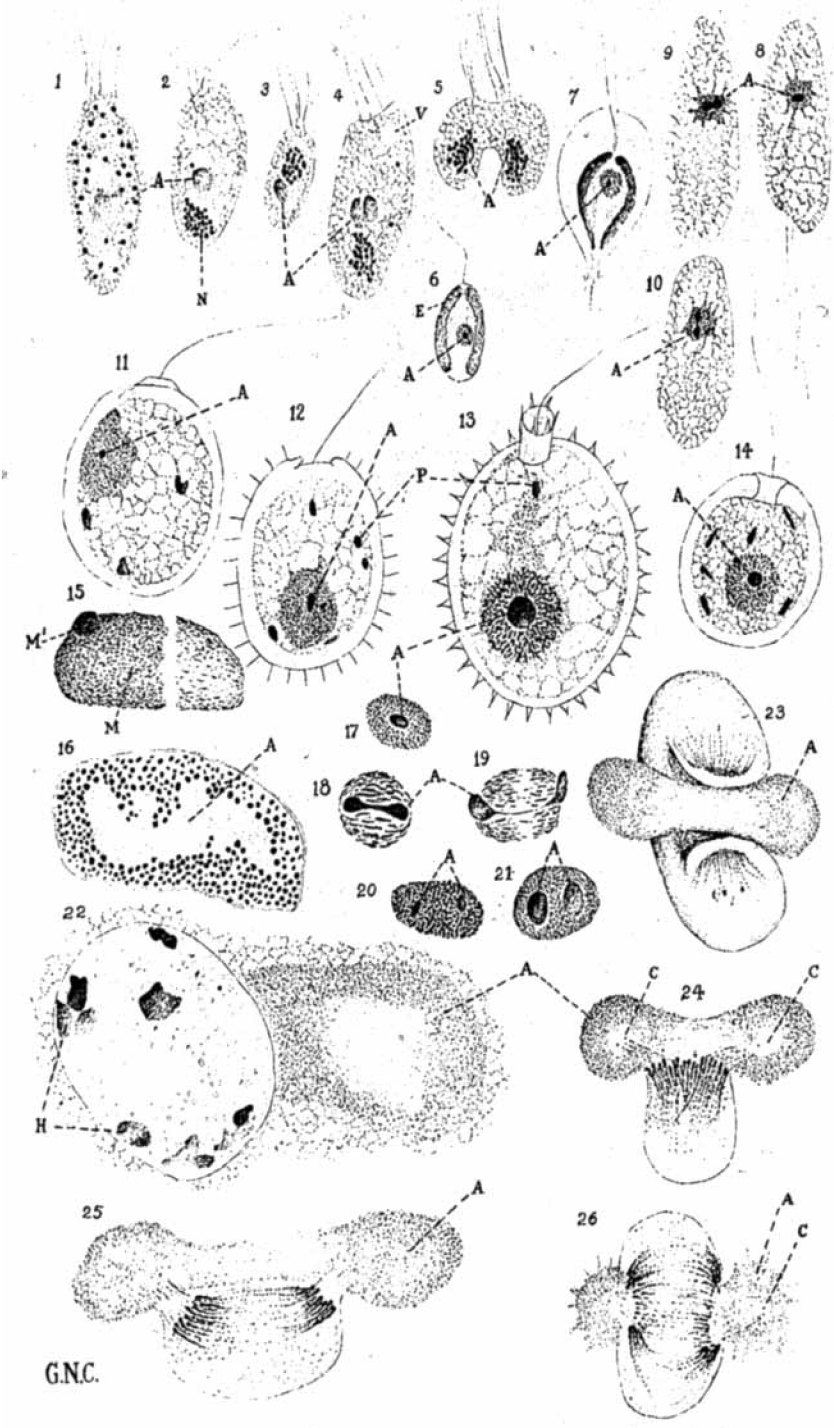
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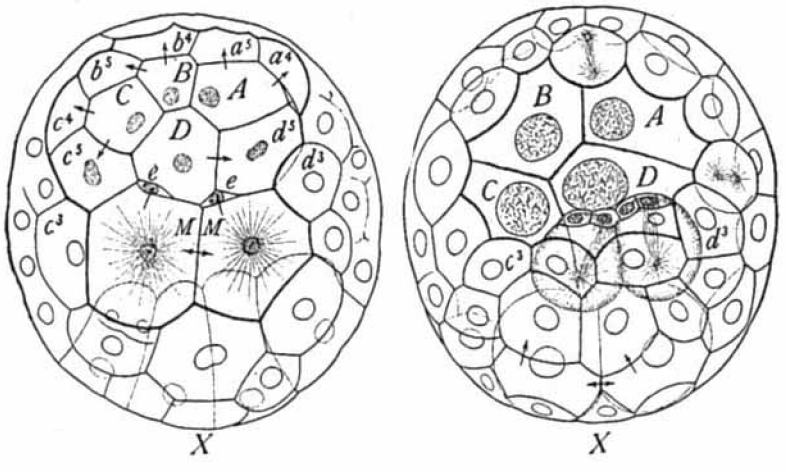
thus formed some of the small cells usually extend, appearing in sections in the sharpest contrast both to the large rounded mesoblast-cells and to those of the lateral ectoblast (Fig. 3, *C*). From this point the mesoblast-bands extend towards the sides and ultimately curve upwards (forwards with respect to the adult long axis) at the sides of the embryo.[1]



A *B*

FIG. 2. Corresponding surface views, from the lower pole, of early embryos of *Aricia* (***A***) and *Nereis* (***B***); the limit of the ectoblast, *i. e.*, the lip of the blastospore, is shown by the heavy line. ***A*** shows the single pair of vestigial entoblasts (*e*, *e*) of *Aricia* lying in front of the primary mesoblasts which are dividing to form the mesoblast-bands (cf. Fig. 1, ***C***, which shows the same specimen in sagittal section). ***B*** shows two pairs of superficial entoblasts, lying behind the macromere *D*, and the spindles of a deeper budding of the "primary mesoblasts" (cf. Fig. 3, ***A***, for section of this stage).

A, *B*, *C*, *D*, the four basal entoblasts or macromeres; a^4–d^4, the fourth quartet of "micromeres" (entomeres); a^5–d^5, the fifth quartet (entomeres); c^3–d^3, derivatives of the third quartet (ectomeres); *M*, *M*, the primary mesoblasts (shaded in ***B***).

Up to this point the account here given is substantially the same as that contained in my earlier paper on *Nereis*. Regard-

[1] In *Aricia* the mesoblast-bands are formed much earlier, while the primary mesoblasts still lie at the surface (Fig. 1, *C*); and they lie at first side by side, nearly parallel to each other, extending upwards behind the entoblast-plate (Fig. 7). In both these respects *Aricia* is somewhat similar to *Lumbricus* (Cf. Wilson, Embryology of the Earthworm, Fig. 30: *Journ. Morph.*, 1889).

ing the fate of the small cells, however, my first account was wide of the mark; for I believed that they migrated into the interior and spread out upon the walls of the archenteron to form a part of the splanchnic mesoblast.[1] I accordingly called the small cells "secondary mesoblast" and applied the same term to the rudimentary cells of *Aricia* and *Spio*. Later studies by several observers seemed to confirm this conclusion. Lillie found in *Unio* a single pair of small superficial cells, budded forth from the "primary mesoblasts" exactly as in *Aricia* or *Nereis*, but relatively larger, which he likewise believed to wander into the cleavage-cavity to form a part of the mesoblast.[2] Heymons found in *Umbrella* two pairs of corresponding but still larger cells, which he, too, apparently traced into the mesoblast.[3] Mead found that a corresponding pair of minute cells, in *Amphitrite* are carried in at the tips of the mesoblast-bands;[4] while Holmes still more recently states that in *Planorbis* they enter the segmentation cavity.[5] Wierzejski's recent observations on *Physa*,[6] though differing from the foregoing in some important details, agree in referring the small cells, of which several pairs are formed, to the mesoblast. With such an array of confirmatory evidence my original conclusion seemed to be strongly supported. Conklin, however, in his remarkable paper on *Crepidula*, reached a wholly different result, finding in that gasteropod that cells which probably correspond with the small cells of *Nereis*, *give rise to the posterior part of the archenteron*.[7] In regard to *Nereis*, I have long suspected that my original account of the fate of the small cells was erroneous. A renewed examination of the matter has left no doubt that such was the case, and gives the strongest ground for the conclusion that, like the corresponding cells in *Crepidula*, *they enter into the formation of the archenteron*. The evidence for this conclusion is as follows:

In my earlier paper on *Nereis* I overlooked the fact that, besides the small cells derived from the "primary mesoblasts,"

[1] *Nereis*, p. 413.
[2] 1895, p. 28.
[3] 1893, p. 281.
[4] 1897, p. 248.
[5] 1897, p. 101.
[6] 1897, p. 389.
[7] 1897, p. 71.

other closely similar cells are formed, just in front of them *by budding from the macromeres.* These cells agree closely with those derived from the "primary mesoblasts" both in size and in the close reticulation and intensely chromatic character of

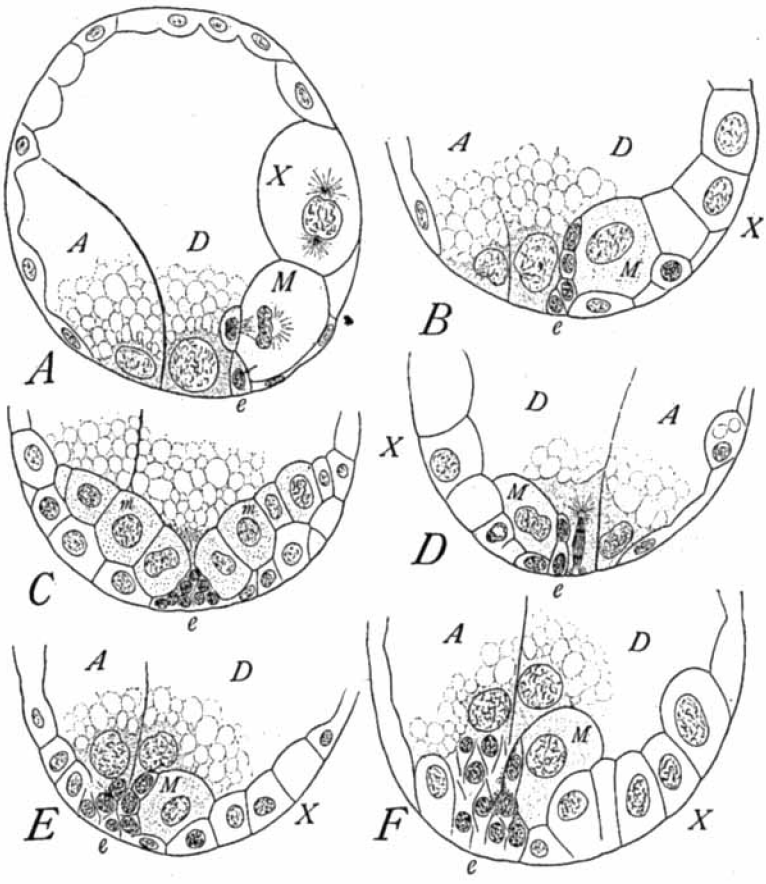


FIG. 3, NEREIS. Sections of successive stages in the formation of the entoblast-plug and mesoblast-bands in embryos of *Nereis* (actual sections, Flemming's fluid; **C** is tranverse, the others sagittal). Lettering as before. **A** shows a deep budding of *M* (cf. Fig. 2, **B**); **B**, later stage showing group of small cells (*e*) derived from *M*; **C**, still later stage, nearly transverse, showing the mesoblast-bands (*m*, *m*) and the group of small cells (*e*) below; **D**, budding of the posterior macromere, *D*; **E**, recession of the entoblast-nuclei; **F**, first appearance of the pigment in the small cells.

their nuclei. The first of them to be formed are budded forth at the surface near the lower pole at a time when the "primary mesoblasts" have budded three or four times (Fig. 3, *D*). Those produced later do not reach the surface, the macromere-nuclei receding from the surface and leaving below them (towards the surface) a closely packed mass or plug of small cells (Fig. 3, *E*), the more anterior of which have been derived from the macromeres, and, therefore, are unquestionably of entoblastic origin,[1] while the more posterior have been derived from the "primary mesoblasts." This plug is bordered in front and at the sides by the ectoblast-cells of the lips of the blastopore, which has now become much diminished in size, while posteriorly it abuts superficially against the ectoblast-cells of the somatic plate (derivatives of "d^2" or "X," the first somatoblast) and at a deeper level against the primary mesoblasts (Fig. 3, *E*). In the cells of this plug are now developed coarse granules of black pigment (Fig. 3, *F*), by means of which they are so unmistakably marked that their later history may be followed step by step with great accuracy. Thus arises the pigment-area at the lower pole of the trochophore larva, described in my first paper on *Nereis*.[2]

In that paper I concluded that the pigment-cells were derived solely from the "primary mesoblasts," having overlooked the fact described above that a part of them, and probably the greater part, are derived from the macromeres (entomeres). I reached the further conclusion that the pigment-cells wandered into the interior and spread out upon the wall of the archenteron to form a part of the splanchnic mesoblast.[3] Renewed studies demonstrate the erroneous nature of this latter conclusion, and prove that *the pigment-cells give rise to the posterior part of the archenteric wall itself*. Both in total preparations and in serial longitudinal sections[4] of the successive stages, every step can

[1] These cells are obviously comparable to the entoblast-cells of the fourth and fifth quartets (and later entoblast-derivatives) in other annelids. In *Nereis* they show no definite arrangement.

[2] 1892, pp. 412, 417.

[3] *Nereis*, p. 413.

[4] The best results were obtained with strong Flemming's fluid.

be followed of the progressive inwandering of the pigment-cells (Fig. 4) to form the narrower posterior part of the pear-shaped archenteron, while the anterior part is developed from the four macromeres (entomeres) as is proved by the fact, among others, that the fat-drops are found lying in its wall. There is no possibility of mistaking the fact that the pigment-cells actually form the archenteric wall, for their outlines can easily be seen

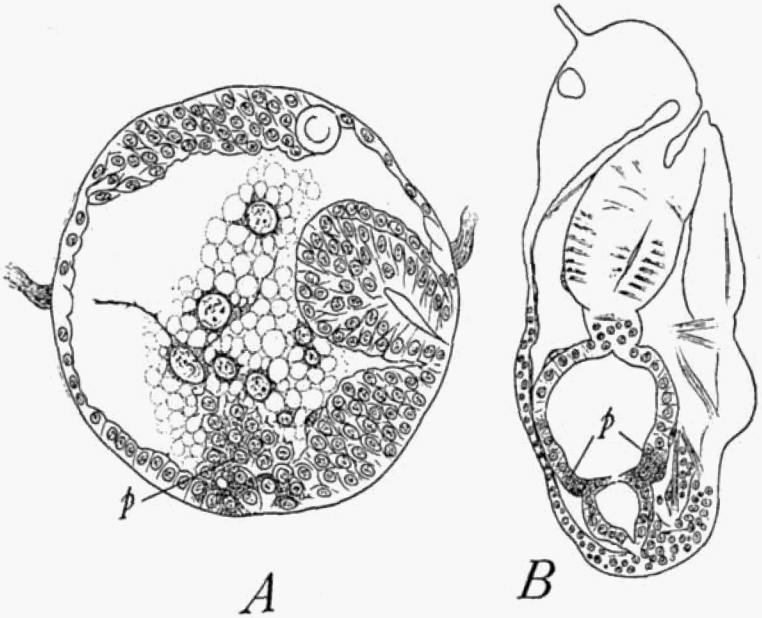


FIG. 4, NEREIS. *Sagittal sections of larvæ.* ***A***, *trochophore (60 hours), showing inwandering of the pigment-cells at the lower pole; stomodæum and neural plate at the right;* ***B***, *larva of 4½ days, showing the pigment-cells at p.*

and the pigment-granules are found throughout the whole thickness of the wall (Fig. 4, *B*). The pigment-cells are, therefore, not mesoblastic, but are *entoblast-cells*.

In so far as the pigment-cells are derived from the macromeres (entomeres), this is exactly what we should expect. That cells derived from the "primary mesoblasts" should enter into the formation of the archenteron is however a surprising result; and it is, therefore, highly important to make

certain, first whether the pigment-cells are in part identical with or descended from the small cells budded forth from the "primary mesoblasts," and second, whether, if this be the fact, the cells of such origin also wander in to form a part of the entoblast. A careful study of the successive stages in surface views, optical sections, and actual serial sections hardly leaves room for doubt in regard to either point. In the first place, pigment is developed in the small cells that abut directly against the primary mesoblasts (Fig. 3, *F*), and the products of the latter form so considerable a group that it would hardly be possible to overlook their displacement or wandering away did such a process occur before the appearance of the pigment. I can find no evidence of such displacement and hence cannot escape the conclusion that the pigment-cells lying just anterior to the primary mesoblasts have been derived from them. The evidence on the second point, while perhaps not demonstrative, is hardly less convincing. The pigment-cells disappear from the surface *pari passu* with the growth of the archenteron; and when the latter is fully formed (in embryos of five days and upwards) not a trace of pigment can be found at the surface or in any of the cells of the posterior region save those of the archenteron. That the superficial pigment-cells actually pass inwards is proved by the fact that from its first appearance the pigment is densest in two (sometimes three) symmetrical areas which are first seen at the surface and may then be traced progressively inwards in the archenteric wall.[1]

Taken together, these facts leave no doubt, in my opinion, that the pigment-cells are derived in part from the primary mesoblasts, in part from the entomeres, and that the cells from both sources give rise to a portion of the archenteric wall and to no other structure. If this conclusion be correct, it follows that the "primary mesoblasts" are not properly so-called, but are *mesentoblasts*, precisely as Conklin has described in *Crepidula*. Now, there can be no doubt that the single pair of minute cells in *Aricia* and *Spio* represent the group of cells of like origin in

[1] Cf. 1892, Figs. 79-91, which show this fact, though not as clearly as it appears in my more recent preparations.

Nereis. They must, therefore, be regarded as *vestiges of functional entoblast-cells such as those of Nereis, and morphologically they represent the posterior part of the entoblast-plate*[1] (Cf. Fig. 1, *B*; Fig. 2, *A*).

The foregoing interpretation is entirely in harmony with Conklin's important discoveries in the gasteropod *Crepidula*. Conklin here definitely showed, for the first time in any animal,[2] that the so-called "primary mesoblasts" give rise to a group of entoblast-cells before dividing to form the mesoblast-bands. But more than this, *Crepidula* represents a step in the series which may be regarded as anterior to the condition found in *Nereis*; for here each mesentoblast divides off two entoblast-cells, the bulk of which taken together is actually greater than that of the mesoblastic material remaining, "less than half the cell (4d) being destined to form mesoblast."[3] The three forms *Crepidula, Nereis, Aricia*, thus form a progressive series in which the entoblastic part of the mesentoblast cell is reduced from more than half the bulk of the cell to an insignificant vestige. It is probable that two intermediate steps besides *Nereis* have been observed by Lillie and Mead respectively. The two cells found by the first named observer, in *Unio*, are somewhat larger than those of *Nereis*;[4] while in *Clymenella* as described by Mead, they are equal in size to the mesoblastic moiety.[5]

[1] It would be interesting to determine whether the vestigial cells of *Aricia* may not be taken into the archenteric wall and thus still retain their functional significance. I have not thus far been able to determine this point; but Mead's observations on *Amphitrite* seem to show that in this form such is not the case, for the vestigial cells are here formed so far from the surface that they pass into the cleavage-cavity and are carried forwards at the tips of the mesoblast-bands. Mead himself concludes that their position in *Amphitrite* is secondary, being a "reminiscence of a surface division which still persists in many forms" (1897, p. 295) I would suggest that their position in *Amphitrite* may be due to the early inwandering of the "primary mesoblasts." It is not surprising that a vestigial cell of this kind should vary somewhat in position; and it should be recalled that in *Nereis* the later-formed cells lie at some distance below the surface. In *Aricia*, too, the vestigial cells do not always reach the surface.

[2] Compare, however, the somewhat similar earlier accounts of Patten for *Patella* (1896) and Stauffacher for *Cyclas* (1893). See Conklin, p. 71.

[3] *Crepidula*, p. 69.

[4] *Unio*, Fig. 60.

[5] 1897, Fig. 88.

Neither of these observers, it is true, suggests the interpretation given above, Lillie somewhat doubtfully assigning to the superficial cells the same fate as I originally did in *Nereis*, while Mead leaves the matter undetermined. It seems probable, however, that we may look for the same fate for these cells as in *Crepidula* or *Nereis*,[1] indeed I venture to think that Lillie's observations are themselves open to such an interpretation.[2]

These facts, I believe, support the view which has been held by many embryologists from the time of Kowalevsky onwards[3] that the primary mesoblasts, or mesoblastic pole-cells of annelids and mollusks must be regarded as derivatives of the archenteron. In both these groups the primary mesoblasts are derived from the posterior cell of the fourth quartet of "micromeres," the lateral and anterior cells of which are, so far as we know, strictly and always entoblastic. The facts indicate, further, that a progressive process of differentiation in cleavage has been going forward, through which the posterior cell of this quartet has become more and more strictly given over to the formation of mesoblast. The vestigial cells of *Aricia*, *Spio*, *Amphitrite* and *Planorbis* would seem to represent the last traces of such archenteric origin of the teloblasts; and it is possible, indeed probable, that there are cases in which even these traces have disappeared, the posterior cell of the fourth quartet being strictly mesoblastic from the first.[4]

[1] Conklin has fully considered (*Crepidula*, p. 72) the apparently contradictory case of *Umbrella*, as described by Heymons (1893), where cells exactly corresponding to the "posterior enteroblasts" of *Crepidula* are described as giving rise to mesoblast. Despite Heymon's careful account, I venture to think that the case demands re-investigation in the light of Conklin's work. In a recent account of the mesoblast in *Physa* (1897), Wierzejski finds that small cells ("mesoderm-micromeres") are budded forth not only from the "primary mesoblasts" but also from the larger lateral cells derived from them. All these cells are assumed to be mesoblastic, though their fate was not followed out (1897, p. 391).

[2] *Unio*, Fig. 67.

[3] Cf. Kowalevsky, 1871, p. 30; O. and R. Hertwig, 1881, p. 47. Hatschek, 1888, p. 76; Rabl, 1889, p. 207, and earlier literature there cited.

[4] This point must remain doubtful until renewed investigation shall show whether the superficial budding is ever entirely suppressed; for we cannot safely infer its absence from existing accounts, and I am not convinced that my own statement of their apparent absence in *Polymnia* (*Nereis*, p. 458) may not have rested upon an oversight.

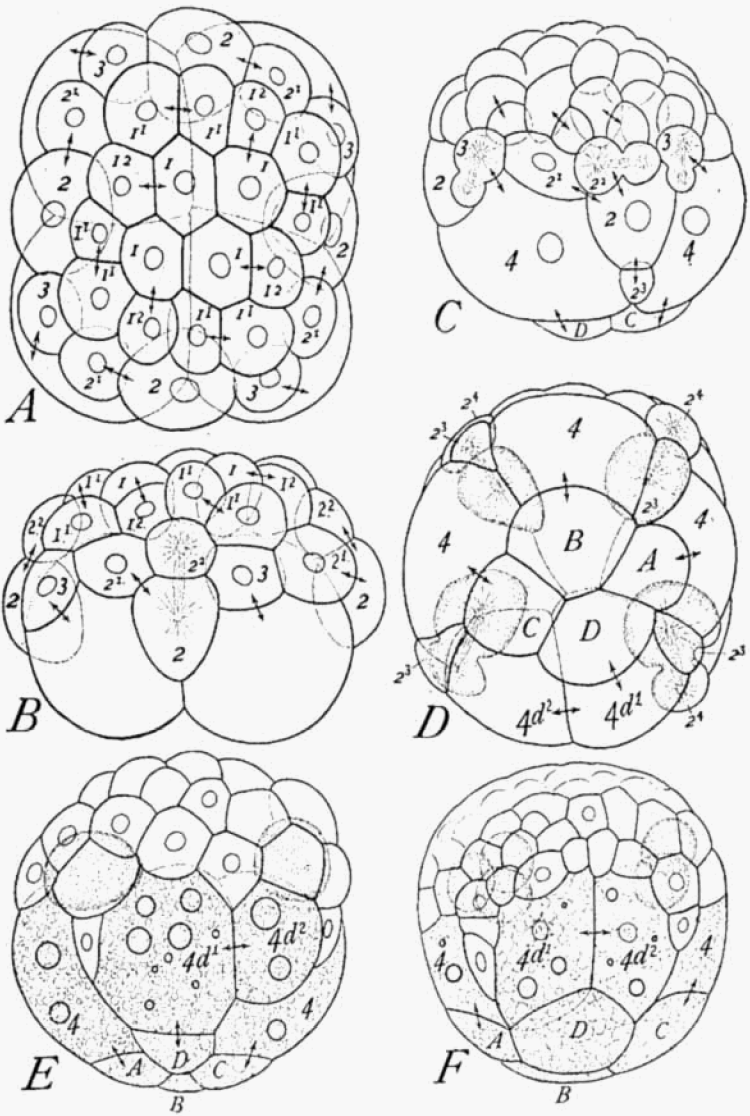


FIG. 6, LEPTOPLANA. (Camera drawings from the transparent living embryos.) ***A***, 32-cell stage, from the upper pole; ***B***, 36-cell stage, from the side, showing second division of 2; ***C***, side view, approximately 60 cells, showing the third ectoblast cell (2^3) derived from 2, the fourth quartet (4) and the basal entoblasts (*D*, *C*). ***D***, delamination of mesoblast in the fourth division of 2 (shaded), from the lower pole, showing the basal quartet of entomeres (*A–D*, and the two somewhat unequal cells ($4d^1$, $4d^2$) formed by the vertical division of the posterior cell of the fourth quartet. ***E***, posterior view of ensuing stage, showing the two posterior mesoblast cells (shaded) lying in the interior, and a marked inequality between ($4d^1$ and $4d^2$). ***F***, later stage; multiplication of the mesoblast-cells (shaded) equality of $4d^1$ and $4d^2$, as in *Discocœlis*.

The bearing of this conclusion on the possible relation between the teloblastic and enterocœlic modes of mesoblast-formation is obvious. This question will, however, appear in a clearer light after a consideration of the polyclade cell-lineage in relation to the foregoing results.

II.

The Micromere-quartets in Annelids, Mollusks and Polyclades.

The marvelously close resemblance in cell-lineage between the annelids, gasteropods and lamellibranchs which recent research, more especially within the last five years, has brought to light, leaves no doubt not only that the general forms of cleavage in these groups are reducible to a common type, but also that a considerable number of more or less definite cell-homologies can be established between them, even in the early cleavage-stages. The attempt to extend the comparison beyond the limits of these groups has, however, thus far encountered a very serious stumbling-block in the cell-lineage of the polyclades. If we accept Lang's view, which is supported by a large amount of evidence, that the platodes are not very far removed from the ancestral prototype of annelids and mollusks, we should expect to find in the polyclade a mode of cleavage to which that of the higher forms can in its main features be reduced. In point of fact, however, this seems to be the case only in the *form* of cleavage and not, so to speak, in its *substance;* for, although the general type of cleavage and the arrangement of the blastomeres in the polyclade shows an extraordinary resemblance to that of the annelid or gasteropod, the cells seem not to have the same morphological value. I have elsewhere sufficiently indicated the nature of this difficulty,[1] which has also been remarked by a number of other writers; but for the sake of clearness I will again direct attention to its leading features.

[1] *Nereis*, p. 441; The Cell, pp. 314, 315.

In the typical development[1] of all the forms in question—polyclades, annelids, gasteropods, lamellibranchs—the egg first divides into four quadrants. From these at least three, and sometimes four or five regular quartets of cells—usually smaller, and hence designated as "micromeres"—are successively produced by more or less unequal and oblique cleavages toward

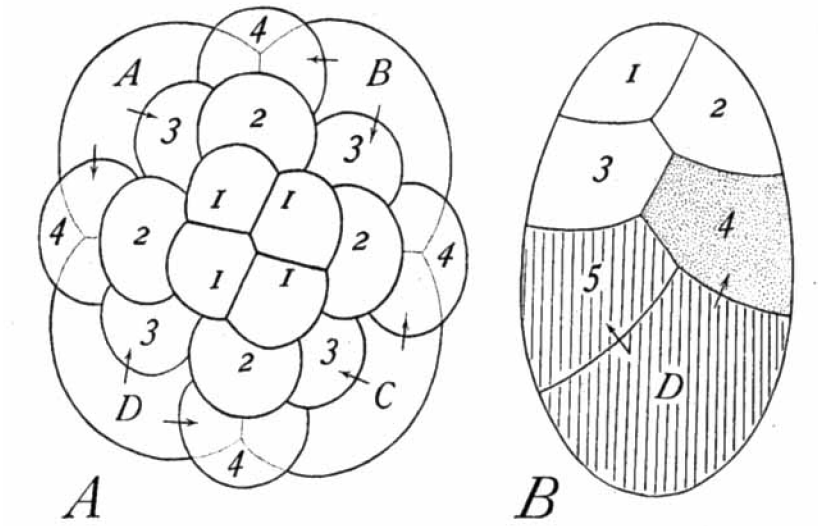


FIG. 5. Diagram showing the typical arrangement of the micromere-quartets in polyclades, annelids and mollusks (their secondary divisions being omitted). ***A***, from the upper pole. ***B***, diagram of the typical history of the posterior quadrant of an annelid or gasteropod embryo; ectoblast is derived from the unshaded cells (1, 2, 3), the mesoblast-bands from the dotted cell (4), ectoblast from the lined cells (5, ***D***).

the upper pole (diagram, Fig. 5). These quartets are displaced according to a definite law, the first being rotated, as it were, towards the right (clockwise), the second towards the left (anti-clockwise), the third to the right, and so on in regular alternation.[2] The secondary divisions of these micromeres also

[1] There are some well-determined exceptions to this mode of cleavage, and at least one of these—the case of *Polycharus*, as described by Gardiner, 1895—is apparently irreducible to it.

[2] The reversal of the direction of displacement in the sinistral gasteropods, discovered by Crampton, is an exception which emphasizes the rule.

show a remarkable similarity, in all the forms, up to a certain point. In morphological value, however, the micromere-quartets of the polyclade appear to differ radically from those of the annelid-mollusk type. In the former the first quartet is described as giving rise to the entire ectoblast, while the second and third quartets are mesoblastic.[1] In the latter, on the other hand, these same three quartets give rise to ectoblast, while, as stated above, the main mass of the mesoblast is derived from a single cell (the posterior) of a fourth quartet of which the other three cells form entoblast (Fig. 5, *B*). If a fifth quartet is formed it is invariably entoblastic (Fig. 2, *A*).

At the time attention was first called to these differences it seemed hopeless to reconcile them. Later researches showed, however, that the discrepancy was not so great as it seemed. Lillie first discovered in 1895 that in the lamellibranch *Unio* one cell (the left) of the second quartet give rise to mesoblastic elements (the "larval mesenchyme")[2] and more recently Conklin has found a similar derivation of mesoblast-cells from three cells (right, left and anterior) of this quartet in the gasteropod *Crepidula*.[3]

It is clear that these interesting discoveries partially bridge the gap between the polyclade and the other forms; though how great it still remains may be judged from the fact that Conklin still regarded the differences as "very great, perhaps irreconcilable,"[4] while Mead, in a still more recent work on the cell-lineage of annelids, is forced into a position of skepticism regarding Lang's whole account of the origin of mesoblast in the polyclade.[5]

For these and other reasons a re-examination of the early development of polyclades has become in the highest degree desirable. After a search extending through several years, I have at length succeeded in finding a form very favorable for this purpose—a species of *Leptoplana*[6] having eggs that are large

[1] Lang, 1884.
[2] *Unio*, p. 24.
[3] *Crepidula*, p. 150.
[4] *Crepidula*, p. 196.
[5] 1897, p. 289.
[6] An undetermined species found in great profusion at Port Townsend, Washington, on Puget Sound.

and transparent, are easily procurable in large numbers, and develop so slowly that the successive stages may be very accurately followed in life, while every point may be repeatedly verified in a large number of specimens. The results of a study of these eggs not only help still further to set aside the apparent contradiction between the polyclade and the annelid-mollusk type, but, when taken in connection with the foregoing observations on annelids and gasteropods, also raise some highly interesting questions regarding the relation of cell-lineage to ancestral reminiscence.

I shall not here describe the cleavage of *Leptoplana* in detail, but will only indicate its leading features. Up to the thirty-two-cell stage, and for some distance beyond, the cleavage is a most beautiful example of the symmetrical spiral type, agreeing very exactly with *Discocœlis* as described by Lang, excepting in the fact that in the four-cell stage the cross-furrow is inconstant and often wanting. The first three quartets of micromeres are formed exactly as in an annelid, and have the same position and relative size as in *Discocœlis* (Fig. 5, *A*), while the four large cells remaining give rise to the archenteron. Regarding the morphological value of these three quartets, however, my results differ very considerably from Lang's and are such as to bring the polyclade cell-lineage into direct relation with that of the annelid, gasteropod and lamellibranch. As in these groups *all three of the quartets give rise to ectoblast*, the first and third apparently to ectoblast alone, though I am not certain that the third quartet may not give rise also to a small modicum of mesoblast-cells. The principal interest centers in the second quartet, from which, as Hallez, Götte and Lang have shown, the principal mass of the mesoblast is formed. What these observers have failed to observe is the fact that each cell of this quartet gives rise to several ectoblast-cells—at least three, and probably four—before sinking into the interior to form mesoblast. These divisions are of constant form, as follows: During the fifth cleavage each cell divides unequally towards the left as viewed from the side (*i. e.*, clockwise, as seen from above) to form an ectoblast-cell ("2^1") that abuts against a

cell of the third quartet formed about the same time (Fig. 6, *A*).[1] The second division is nearly or quite horizontal, separating a second ectoblast-cell ("2^2") directly above the original or stem-cell (Fig. 6, *B*). The third ectoblast cell ("2^3"), which is very small, is budded forth at the lower tip in the angle between the macromeres (Fig. 6, *C*, *D*). The three cells thus formed (2^1, 2^2, 2^3, Fig. 6) enter, as I believe, into the general ectoblast. At the fourth division the stem-cell divides unequally in a direction parallel to the surface, a large inner cell being delaminated off from a smaller superficial cell (2^4, Fig. 6, *D*). *The inner cell is forced into the angle between the two adjoining "macromeres," and forms one quadrant of the mesoblast; the outer cell flattens out at the surface and is, I believe, an ectoblast-cell*, though I am not entirely sure that it may not ultimately migrate into the interior to form mesoblast. The four primary mesoblast-cells thus formed rapidly multiply to form four groups of rounded granular cells (Fig. 6, *F*) which may easily be seen for a long time through the transparent ectoblast and from which the greater part, if not all, of the adult mesoblast is derived.

It is clear from these facts that the cells of the second quartet in the polyclade (*i. e.*, in *Leptoplana*) are not purely mesoblastic, but are *mesectoblasts*. It seems equally clear that the formation of "larval mesenchyme" from certain cells of the second quartet in *Unio* and *Crepidula* must be regarded as an ancestral reminiscence or survival of the process that occurs in all four of the cells in the polyclade, and it is an interesting question whether such a survival may not also occur in the embryos of annelids. A careful re-examination of *Nereis* with respect to this point has thus far yielded a negative result. In *Aricia*, on the other hand, it is probable that two mesoblast-cells arise from either the second or third quartet, though the material at my command has not enabled me to reach a decisive result. At the stage shown in Figs. 1, *C*, and 2, *A*, two large and very conspicuous rounded cells are found lying, one on either side, in the cleavage-cavity between the lateral ectoblast and the mesoblast-band (*y*, *y*, Fig.

[1] Lang figures this division—Pl. 35, Fig. 5.

7) and slightly anterior to the latter. Sections show that these cells are budding forth smaller cells into the cleavage-cavity. I am nearly certain that these cells are not derived from the entoblast; and their position is such that an origin from the primary mesoblasts is improbable. They are often closely wedged in between the overlying ectoblast-cells, and all the appearances indicate that they have been derived from the latter. From their position I believe it probable that these cells have been derived from the two lateral cells of either the third or the second

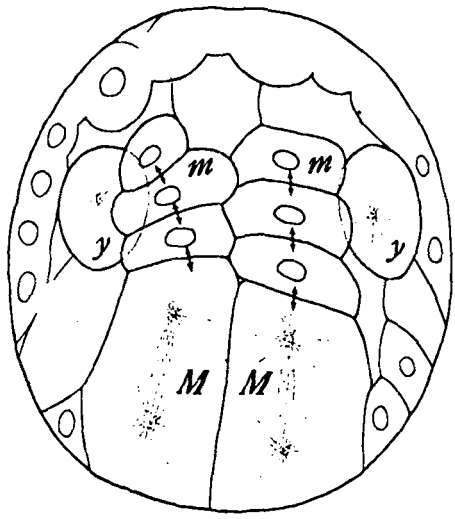


FIG. 7, ARICIA. Frontal optical section[1] of early embryo of *Aricia*, showing the parallel mesoblast-bands (*m*, *m*) extending upwards from the primary mesoblasts, *M*, *M*, behind the entoblast plate (cf. Figs. 1, **C** and 2, ***A***, which show the same individual in different positions). At the sides of, and slightly anterior to, the mesoblast-bands are the two mesoblast-cells (*y*, *y*) of probable ectoblastic origin.

quartet—*i. e.*, from derivation of c^3 and d^3, or of c^2 and a^2 (*Cf.* Fig. 2, *A*)—and that they accordingly are comparable to the "larval mesenchyme" or "secondary mesoblast" (*i. e.*, the ecto-mesoblast) of *Unio* and *Crepidula*. Future investigation must determine whether this surmise be correct, and what is the ultimate fate of these cells, but the facts give, I think, good reason

[1] Confirmed by actual sections.

to expect that the annelids will ultimately be shown to agree with the mollusks in showing reminiscences of the ancestral mode of development in the double origin of the mesoblast.

Returning now to the mollusks, Wierzejski, in a recent preliminary paper (1897) states very explicitly that in *Physa* a part of the mesoblast is derived from two cells of the *third* quartet.[1] This result, if well founded, gives good reason to suspect that the third quartet may give rise to mesoblast in some of the polyclades, as Lang has maintained for *Discocœlis*. In *Leptoplana* I have sought carefully for evidence of such a process, but thus far without success. This negative result is, however, inconclusive owing to the difficulty of tracing the later history of the individual cells. The first division of the third quartet is vertical to the surface (Fig. 6, *C*) and in later stages I have thus far found no evidence that a delamination of mesoblast occurs. Soon after the delamination of mesoblast in the second quartet, all of the ectoblast-cells forming the lips of the blastopore become much flattened (Fig. 6, *F*), while the ectoblast-cap rapidly extends downward, the blastopore finally closing at or near the lower pole. In these stages the outlines of the thin ecoblast-cells are very difficult to see, either in life or in preparations, owing to the confusion produced by the underlying deutoplasm-spheres, now much increased in size, on which they are moulded. The mesoblast now forms four groups of rounded granular cells conspicuously seen through the transparent outer cells. A study of the successive stages proves that the greater number of these are derivatives of the second quartet; but the possibility remains that some additions may have been made from the third quartet.

From the foregoing account it appears that the "mesoblast" of the polyclade is derived from the ectoblast; and it may, I think, be taken as a fair working hypothesis that this "mesoblast" is represented in the mollusks, and probably also in some annelids by cells ("larval mesenchyme," etc.) derived from the second quartet (*Unio*, *Crepidula*, *Aricia* (?)) or perhaps in

[1] Confirmed by Holmes in the case of *Planorbis* since the above was written. See *Science*, VI, No. 154.

some cases from the third quartet (*Physa*, *Aricia* (?)).[1] Assuming this to be the case, what shall we say of the mesoblast-bands, which are in annelids and mollusks derived from the fourth quartet and which, as we have seen reason to conclude (p. 12), are probably to be regarded as derivatives of the primitive archenteron? The development of the polyclade suggests an answer to this question which is in harmony with the facts discussed in the first part of this paper. As earlier observers have shown, the fourth division of the "macromeres" in the polyclade is unequal, giving rise to four smaller cells *at the lower pole* of the embryo (*A–D*, Fig. 6, *C–E*), and to four much larger cells lying above them. From these eight cells, which are heavily laden with deutoplasm and differ entirely in appearance from the ectomeres and mesomeres, the archenteron is formed. With this *Leptoplana* exactly agrees, and I can find no evidence that mesoblast-cells are formed from any of these eight cells. If now we judge solely by relative position without respect to size, the four larger cells or "macromeres" (4–4) correspond exactly with the fourth quartet of annelids and mollusks—in fact, they are relatively not very much larger than in some of the mollusks (*e. g.*, *Planorbis*, *t.* Rabl, 1880). Lang discovered the remarkable fact that in *Discocœlis*, as in so many of the latter animals, the posterior cell of these four divides long before the others; and further, that this division is equal, giving rise to two symmetrically placed cells at the posterior end of the embryo, while the ensuing divisions of the other three cells of the quartet are unequal and irregular.[2] Mead[3] has pointed out the very remarkable resemblance of these two cells in *Discocœlis* to the "primary mesoblasts" of annelids and gasteropods and even goes so far as to suggest that they may give rise to mesoblast-bands in the polyclade. My observations on *Leptoplana* lend no support to this suggestion, agreeing nearly with those of Lang on *Discocœlis* save in

[1] Edouard Meyer (1890, p. 299) has definitely compared the "parenchyma" (mesoblast) of the Turbellaria with the "larval mesenchyme" of the annelids, which he believes to have a different origin from the mesoblast-bands.

[2] Cf. Lang, 1884, Figs. 17–20.

[3] 1897, p. 289.

one noteworthy respect, namely, that the division of the posterior "macromere" is variable, only rarely dividing equally (Fig. 6, *F*) and as a rule dividing unequally, giving rise to a smaller cell ($4d^2$, Fig. 6, *E*) that is typically formed obliquely towards the right as seen from the side (*i. e.*, in a leiotropic or anti-clockwise spiral.[1] From this it appears that the form of cleavage in the fourth quartet of *Discocœlis*, which agrees so exactly with that of the annelids and mollusks, appears as only an occasional variation in *Leptoplana*, though even here the posterior "macromere" is always the first to divide.

As regards the fate of these cells, the inequality of $4d^1$ and $4d^2$ (often very marked) is itself indirect evidence that they do not give rise to symmetrical mesoblast-bands as in the higher types and I find no evidence that either of them gives rise to mesoblast-cells. Both seem to have the same fate as the other entoblast-cells, with which they exactly agree in deutoplasmic structure, and enter into the formation of the archenteron as Lang has shown in the case of *Discocœlis*. Can we nevertheless regard them as homologous to, or rather as the prototypes of, the primary mesentoblasts of the annelids and mollusks? When we reflect on the facts, reviewed in the first part of this paper, we may hesitate to answer this question in the negative. For we have seen reason for the conclusion that the primary mesoblasts of annelids and gasteropods have arisen historically, as they arise ontogenetically, *from the posterior part of the archenteron;* and we have traced the entoblastic elements of the posterior cell of the fourth quartet from a minute and apparently functionless vestige (*Aricia*) back to a group of large and important cells (*Crepidula*). I think we should consider the possibility, if only as a working hypothesis, that in ancestral types the entoblastic elements of the posterior cell of the fourth quartet

[1] Typically—*i. e.*, in probably ninety per cent. of the cases observed, the division is markedly unequal—often much more so than in Fig. 5, *E*. In a few cases the direction of division is reversed, the smaller cell, $4d^2$ being found towards the left (dexiotropic spiral). Sometimes the division is equal and vertical as in *Discocœlis;* more rarely it is horizontal and either equal or unequal. I believe all these variations occur in normal embryos. A considerable time after the formation of $4d^2$ the other macromeres begin to divide unequally and irregularly, and all the macromeres ultimately break up into smaller rounded cells, heavily laden with deutoplasm.

may have preponderated as greatly over the mesoblastic as the latter now preponderates over the entoblastic in *Aricia;* and that the beginning of the series may have been such a mode of development as still occurs in the polyclade where the entire quartet is entoblastic. Thus we are brought anew to the view which has been advocated by a number of morphologists, prominent among them Edouard Meyer,[1] that the mesoblast-bands (entomesoblast) of the higher forms may have been of different origin phylogenetically from the "larval mesenchyme"[2]

More specifically I would suggest that in the ancestral type the fourth quartet was strictly entoblastic; that at a later period in the phylogeny the trunk-mesoblast (mesoblast-bands of higher types) took its origin from the posterior part of the archenteron, perhaps in connection with the development of a new body-region from the posterior part of the ancestral body; and that as the cleavage became progressively specialized (*i. e.*, assumed more of what Conklin has termed a "determinate type") the seat of this mesoblast-formation became more and more definitely localized in the posterior member of the fourth quartet. The symmetrical division of this cell in the polyclade might accordingly be regarded as the prototype of that which occurs in the annelid or mollusk, though the resulting cells have in the latter forms acquired a different morphological significance. In other words the old building-pattern, still persisting more or less definitely in the polyclade, has been adapted to a new use[3] precisely as in the evolution of adult structures.

I would distinctly repeat that these suggestions are offered only as a speculative working hypothesis; yet, despite their hypothetical character, it seems to me that they may give a new point of attack upon some of the puzzling phylogenetic problems with which the study of cell-lineage has to grapple.

[1] 1890, p. 299.

[2] † Cf. Conklin, p. 151.

[3] "Imagine that in any species a new organ is added, or rather, that a diffuse series of structures gains great importance and compactness in the course of evolution. Then this new structure *may be* represented in ontogeny by a cell. But the form of cleavage is already defined. * * * The manufacture of a new cell being an impossibility, an old cell must be modified to represent the new organ." (Lillie, 1895, p. 37.)

III.

On Cell-lineage and Ancestral Reminiscence.[1]

The phenomena shown in the history of the micromere-quartets in platodes, annelids and mollusks are, I think, of general interest in two directions.

In the first place they render it highly probable, if they do not actually demonstrate, that development may exhibit ancestral reminiscence as clearly in the cleavage of the ovum as in the later formation of tissues and organs. That the rudimentary entoblasts of *Aricia*, *Spio*, or *Amphitrite* are such ancestral reminiscences seems almost as clear as that the yolk-sac of the mammalian embryo or the primitive streak of a bird-embryo are such; and the same may be said of the formation of mesenchyme-cells from the second quartet in *Unio* or *Crepidula* These facts, among many others, may well give us hope that, when the comparative study of cell-lineage has been carried further, the study of the cleavage-stages may prove as valuable a means for the investigation of homologies and of animal relationships as that of the embryonic and larval stages. The results of experimental embryology have no doubt seemed adverse to such a conclusion, by showing how easily the cleavage-stages may be altered by changes in the conditions of development. But I cannot see that the embryonic and larval stages are in much better case. Certainly the modification of cleavage-forms which Driesch has effected in the echinoderm egg by pressure, temperature and the like, are hardly greater than those which Herbst has brought to pass in the gastrular and larval stages of the same eggs through modification of the chemical environment. It is true that nearly related forms—for example the gasteropods and the cephalopods—may differ very widely in the form of cleavage; but so they may in the embryonic and larval stages, and it may fairly be questioned whether "secondary modification" or "cœnogenetic change" has gone further in one case than in the other.

[1] The term "ancestral reminiscence" is here used to denote any feature of development, the meaning of which is only apparent in the light of earlier historical conditions, whether of the adult or of the embryo.

Recent advances in the study of cell-lineage have, it is true, raised some new apparent difficulties in the attempt to establish precise cell-homologies, even between nearly related forms[1] though I suspect that some of these will be found less serious than they now appear. Against these difficulties, however, may fairly be placed an increasing body of affirmative evidence,[2] and on this side may be ranged the observations recorded in the present paper. We should, moreover, remember that just as the homologies of adult parts may be complete or incomplete in various degrees (as Gegenbaur long since urged), so cell-homologies may be more or less definite. Furthermore, just as we cannot always find exact equivalents, in related forms, of the several sub-divisions of homologous nerves or blood-vessels or sense-organs, so we need not expect to find exact homologues for all the individual cells throughout ontogeny, The wonder is, indeed, that so many definite cell-homologies have been established. I believe the facts now known demonstrate the inadequacy of Hertwig's too simple conclusion that the definite values of the blastomeres, and hence of the cell-homologies based upon them, are merely an incidental result of the continuity of development,[3] and that they do not leave without support the plea made five years ago in my paper on *Nereis*, for the study of cell-lineage as a guide to relationship.[4]

In the second place, these facts seem on the whole to emphasize the importance of cell-formation in development. The inadequacy of the cell-theory as applied to development has been very ably urged, especially by Whitman and by Adam Sedgwick; and their conclusions, fortified by the epoch-making discoveries of Roux, Driesch and others on the development of isolated blastomeres, are of an importance that we are only beginning fully to realize. But the time has not yet come for a just estimate of the cell-theory in this aspect; and it may well be questioned whether in the reaction against the cell-mosaic theory, as originated by Schwann, and developed with so much

[1] Cf. Mead, 1897, and Child, 1897.

[2] Cf. Conklin, 1897.

[3] Cf. the very effective criticism of Conklin, 1897, p. 191.

[4] 1892, pp. 367, 455.

ingenuity by Roux and Weismann, the pendulum of opinion may not have swung too far towards the opposite extreme. The persistence in cleavage of vestigial cells (such as the rudimentary enteroblasts of *Aricia*), or of vestigial processes in the formation of the germ-layers (as in the origin of the "mesenchyme" in *Unio* or *Crepidula*) adds to the evidence that the number and character of the cell-divisions stand in some direct and important relation to the differentiation-process; and it would be difficult to explain such ancestral reminiscence in cell-lineage under any view which does not recognize in cell-outlines the definite boundaries of differentiation-areas in the developing embryo.[1] The history of the posterior cell of the fourth quartet in annelids and gasteropods gives a clue to the process through which teloblasts and other determinate protoblasts have arisen by progressive specialization; and I think it lends support to the distinction drawn by Conklin[2] between "determinate" and "indeterminate" types of cleavage by showing some of the steps by which the former may have been acquired.

From a physiological standpoint the persistence of rudimentary cells in cleavage is a problem of high interest which merges into the larger problem of ancestral reminiscence in general. When one considers the analogous case of the polar bodies, one is almost tempted to suspect that the formation of the rudimentary enteroblasts may be in some way connected with a definite transformation of the nuclear substance. It is, however, equally possible that the removal of the *cytoplasmic* substance of these cells may be a necessary condition of the differentiation of the mesoblastic material.

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December 4, 1897.

[1] Cf. Wilson, 1893, p. 14.

[2] 1897, p. 190.

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