

Some Points in the Metamorphosis of *Asterina gibbosa*.

By

Seitaro Goto,

Professor in the First High School, Tokyo.

With Pl. XVIII.

In my paper on the metamorphosis of *Asterias pallida* published a short time ago ['98 a] I have given expression to views, which are in some points irreconcilable with those of previous observers; and especially was this the case with regard to the observations of MacBride on *Asterina gibbosa* recorded in his excellent paper published in the Quarterly Journal of Microscopical Science ['96]. To decide if these contradictions are only apparent or real and also to extend my observations to some species undergoing a different form of metamorphosis, I asked for the purchase of developmental stages of *Asterina gibbosa* from the Zoological Station of Naples; and although, owing to the lateness of the season, I could not obtain fresh specimens fixed according to my prescription, the authorities of the Station kindly sent me a complete series of eggs and larvæ that had been fixed in a sublimate-acetic mixture. The present paper embodies the results of my observations on this material. I shall not, however, go over the entire ground anew, but will

confine my descriptions to those points on which the statements of other writers require either confirmation, correction or extension.

As the rapidity of development appears to be subject to considerable variation I shall, in the present paper, adopt the notation of MacBride ['96] for the stages of metamorphosis, in which these are represented by the first letters of the alphabet. Thus, stages A, B, C, D, E, F and G correspond respectively to those of the 2nd day, 3 days, 6 days, 5 days, 8 days, 9 days, and 10 days of Ludwig, the last stage representing the first completion of the star. In the case of my specimens the same stages extend over a period of 20 days, and thus it seems that their development was half as fast as those of Ludwig. This discrepancy can be conveniently avoided by adopting MacBride's notation, which has the further advantage of allowing intermediate stages to be denoted in the form of a fraction. Thus in the following pages I shall denote the stage intermediate between B and C stage B/C.

1. External Feature.

In the first place a few words must be said about the orientation of the adult starfish. In *Asterias pallida* I have shown that the plane of bilateral symmetry of the adult coincides with that of the larva, and that the oral side of the former is the anterior of the latter and the aboral side the posterior. We must now examine if the same method of orientation can with propriety be applied to *Asterina gibbosa*, especially as this species has been studied by more than one eminent investigators and none of them has pointed out such a coincidence of the planes of bilateral symmetry of the larva and adult. I have shown that in *Asterias pallida* the remnant of the preoral lobe is, in

a very late stage of metamorphosis, found on the right side of the body. This is brought about apparently by the greater atrophy of the right side of the preoral lobe. In *Asterina gibbosa* this unequal atrophy of the two sides of the preoral lobe becomes manifest in a much earlier stage, when the lobe has scarcely undergone any shrinkage. As a consequence of this the preoral lobe is bent towards the right side of the body, and the aboral disc never makes a right angle with it, as it does in *Asterias pallida*. This is very evident in the figures of Ludwig and MacBride, and is also shown in fig. 14, Pl. XVIII, of the present paper. The maximum inclination of the aboral disc with respect to the preoral lobe is, in the present species, 60° - 70° , as has been pointed out by MacBride, and as may be seen in fig. 13, *c* & *d*. From this maximum inclination on, the lobe makes less and less an angle with the aboral disc, inasmuch as it is bent more and more towards the right side of the body as metamorphosis progresses (fig. 14). But does this show the method of orientation developed in my paper on *Asterias* to be inapplicable to the present case? I think not. Because exactly the same process takes place, as I have remarked above, in *Asterias*, but in a much later stage. If we look closely into the matter we shall see that there are here two forces at work in different directions, one tending to bring the aboral disc and the preoral lobe to such a position that they make a right angle with each other, and the other tending to diminish the angle between them. In *Asterias pallida* the latter force comes into play only at a very late stage of metamorphosis; but in *Asterina gibbosa* its effect becomes pronounced at an earlier stage, and thus disguises the real state of things that would otherwise be manifest. In both cases the plane of bilateral symmetry is bent on itself at the

boundary between the disc and the preoral lobe as soon as the force just mentioned comes into play. The only difference between the two species under consideration is that, in *Asterias pallida* the portion of the plane of bilateral symmetry belonging to the preoral lobe is very insignificant and can be neglected for practical purposes, whereas in *Asterina gibbosa* the preoral lobe is still very large when it begins to be bent. It seems to me that the number of cases where the plane of symmetry undergoes secondary distortion is too numerous to weaken the explanation here offered. I therefore look upon my method of orientation as still valid.

I may add a word on the histology of the preoral lobe. Previous observers have shown that the larvæ are able to stick to external objects by means of the preoral lobe, and MacBride has published a figure [’96, pl. 27, fig. 136] of a section through a larva sticking to an external object by means of a mass of mucin; but nobody has, so far as I know, shown where the glands that secrete the mucin are, nor what their structure is. I find in my sections that there are innumerable unicellular glands not only in the “preoral pit” but also all over the anterior face of the preoral lobe, and especially concentrated along its anterior margin. These cells are either goblet-shaped and have long necks, or are simply tubular and more or less winding; their contents consist of granules which stain deeply with Kleinenberg’s hæmatoxylin and Bismarck brown, so deeply indeed that it is very difficult to detect their nuclei. These cells lie between the tall ectodermal cells of the preoral lobe, and their openings on the surface of the cuticle are very distinctly visible. It is needless to add that the granular contents give rise to the mucin.

2. Hydro-Enterocœl.

In treating of this system I start with stage B/C, figs. 1 & 2, Pl. XVIII. As may be gathered from these figures there are, in this stage, only two cavities entirely separated from each other, one being the left posterior enterocœl and the other including the hydrocœl, the anterior enterocœl and the right posterior enterocœl, all these latter freely communicating with each other (fig. 6). There is no natural boundary between the anterior and the right posterior enterocœl; but I conceive it to be determined by a transverse plane passing through the pore-canal, as I have done in the case of *Asterias*. We also see that the pore-canal lies at the posterior extremity of the anterior enterocœl, on the left side. As implied in the statement made above, the left and the right posterior enterocœl are entirely separated from each other, there being a mesentery on the dorsal as well as on the ventral side (fig. 5). In stage C/D this separation becomes incomplete, the mesentery disappearing for a short extent (between lines *a* & *b*, fig. 4; fig. 7 *b*) on the dorsal side, and on the ventral side as far posteriorly as line *c* (fig. 4). At this stage, therefore, all the cavities communicate with each other directly or indirectly. In stage D the communication on the dorsal side still exists, but the two cavities are again completely separated from each other in the next stage (E, fig. 11); while the mesentery on the ventral side gradually disappears. This process of disappearance of the primary ventral mesentery begins in stage C and is accompanied by the formation of another mesentery on the right side of the body. This secondary mesentery always runs obliquely to the long axis of the larva, as has been correctly figured by Ludwig ['82, figs. 31, 32], and is formed by the

apposition and subsequent transverse separation of the two peritoneal walls, as shown in figs. 3 & 8. In fig. 7 *b* we see the primary mesentery on the ventral side still present, and at a short distance from it on the right side, a new septum is being formed. In *Asterias pallida* I was not able to see these two septa in a similar condition, but in the present species the process of formation of the one as well as that of disappearance of the other can be followed step by step in many sections, one of which I have also reproduced in my preliminary paper on this subject [’98 *b*]; and no doubt can, therefore, be entertained as to the exact extent of the two posterior enterocœls. The cavity formed by the union of the whole left, with a portion of the right, posterior enterocœl I have called the secondary left posterior enterocœl, and the cavity left at the postero-dorsal corner is the epigastric enterocœl; the latter being, as I have stated in my preliminary paper, very nearly one-half of the original right posterior enterocœl. The formation and completion of the secondary mesentery between the epigastric and the secondary left posterior enterocœl proceeds from before backwards, as in *Asterias pallida*.

We have then at this stage two cavities entirely separated from each other; one is the comparatively small epigastric enterocœl, and the other is the anterior enterocœl and the secondary left posterior enterocœl freely communicating with each other. In the next stage (E) the dorsal mesentery dividing the two portions of the secondary left posterior enterocœl is absorbed, and both come to communicate with the anterior enterocœl (*cf.* fig. 11 *d* & *e* and fig. 12 *d* & *e*). The secondary left posterior enterocœl has therefore, at this stage, two passages of communication with the anterior enterocœl, one on the right, and the other on the dorsal, side.

For the sake of continuity we shall follow the changes which the secondary left posterior enterocœl undergoes one stage further, although some important changes are taking place elsewhere. In stage E/F (fig. 13) the passage of communication on the right side just referred to is cut off by the formation of a new mesentery (fig. 13 *d* & *e*), and the passage on the dorsum has also been much constricted, as may be seen in fig. 13 *g*. The separation is effected immediately afterwards, it taking place along a line drawn between a valve-like process next the pore-canal and the posterior extremity of the hydrocœl lobe 5 in the figure just mentioned. When this separation has been effected we have three entirely distinct cavities, the anterior, the epigastric, and the secondary left posterior, enterocœl.

We must now turn back to stage C/D and direct our attention to what has been called the "dorsal sac." According to MacBride ['96, p. 349] this vesicle is budded off from the anterior cœlom at its posterior extremity, and for this reason, as well as from some observations on abnormal larvæ, he regards it as the right hydrocœl. In *Asterias pallida* I stated that this sac was budded off from the left enterocœl directly behind the pore-canal,* and that it was subsequently observed to be an extension of the anterior enterocœl ['98 *a*, p. 254]. In *Asterina gibbosa* I also find the sac originate in exactly the same way, but it remains independent all through its subsequent changes. In fig. 17 I have reproduced four successive sections passing through the dorsal sac, when it is being formed. Although the wall of the dorsal sac is closely appressed to that of the right

*There I added the explanatory words, "that is to say at the boundary between the posterior and anterior enterocœls." This was called forth by the subsequent displacement of the sac referred to in the text, in consequence of which it came to open into the anterior enterocœl.

posterior enterocœl the two remain very distinct, and there is, on the other hand, no ambiguity as to the connection of the sac with the left posterior enterocœl. This series and several more, which it is not necessary to reproduce, decides the question, as it seems to me, beyond doubt. The error of MacBride on this point is perhaps to be attributed to the fact that he studied principally by means of frontal sections and also that his specimens were fixed with osmic acid. The former cut the plane of separation of the two structures under consideration obliquely, and the latter makes, as every one knows, subsequent staining a matter of difficulty. The cases of abnormal larvæ adduced by MacBride in support of his opinion can not, it seems to me, be set off against the observation of normal larvæ.

The subsequent change which the dorsal sac undergoes is, as stated above, different in *Asterias* and *Asterina*. In the former it unites with the anterior enterocœl; in the present species, however, the sac remains, as already mentioned, entirely distinct from other cavities. It undergoes enlargement in subsequent stages, and its wall becomes consequently very thin. Its definitive position in the star is exactly the same as in *Asterias pallida*.

In my former paper ['98, p. 255] I criticised MacBride's statement to the effect that the "dorsal sac" was observed by Cuénot in the adult star directly under the madreporite. I then had the impression that the sac marked *sin'. ax'* in my figures might be something entirely different from the "dorsal sac"; but my observations on *Asterina gibbosa* lead me to conclude that the two structures must be identical.

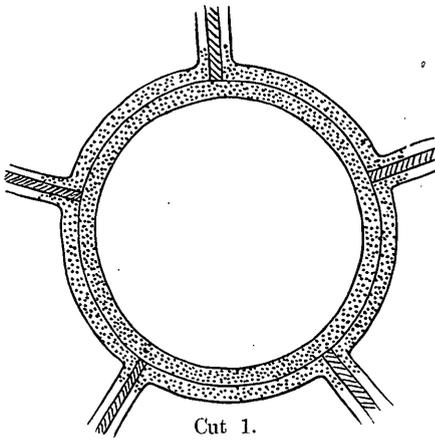
The gradual change of position of the epigastric and the secondary left posterior enterocœl takes place in exactly the

same way as in *Asterias pallida*, as may be followed in the accompanying plate, and will therefore be passed over. The periesophageal enterocœl originates exactly as in *Asterias*, and as described by MacBride. As to the formation of the stone-canal I also find the statement of the author just named confirmed by my observations, which are therefore opposed to those of Ludwig, according to whom it originates from the hydrocœl and grows out towards the pore-canal, with which it finally unites [’82, p. 38].

We shall now pass on to the formation of the perihæmal system, on which I have to record some observations that do not agree with those of MacBride. In the following description I shall retain the words I used in my former paper, and speak of the “circular enterocœl” and the “perihæmal system.” The former is equivalent to the “inner perihæmal ring” of authors and the latter to the “outer perihæmal ring” plus the radial perihæmal spaces. I have shown that in *Asterias* the circular enterocœl is formed from the anterior cœlom and that the whole perihæmal system originates in the form of mesenchymatous spaces. In *Asterina gibbosa* the process is still more complicated, for, anticipating the results of my observations, I may state at once that a portion of the perihæmal system (*mihi*) is found to have an enterocœlic origin. In MacBride’s opinion the entire perihæmal system of authors originates as five interradianl out-pocketings from the secondary left posterior enterocœl. A thorough examination of my series of sections has, however, shown that there are only four such diverticula, and that in the interradius 4/5*, the diverticulum is replaced by the posterior extension of the anterior enterocœl. Diverticula 1/2,

*1/2 of MacBride.

2/3 and 3/4 arise at nearly the same time, viz., between stages E and F, but diverticulum 1/5 is formed for the first time in stage F, and remains considerably smaller than the other three (fig. 14 e). These four diverticula together with the posterior extension of the anterior cœlom do not, however, according to my observations, form the whole perihæmal system of authors, but only the circular enterocœl and the central portion of the



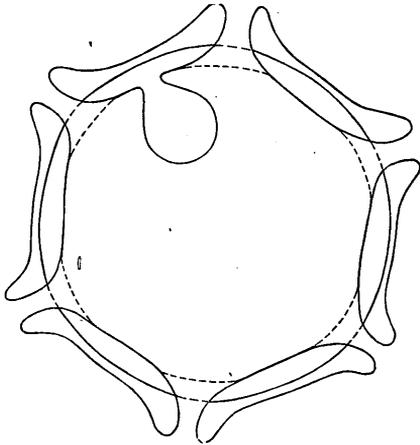
Cut 1.

perihæmal system (*mihi*). In the accompanying cut I have distinguished the enterocœlic portions by stippling; and the portion left vacant arises from the mesenchyme. Let us now follow the changes which the diverticula undergo during metamorphosis, and to do this it will only be necessary to describe the

changes which one of them passes through, as each repeats exactly the same process.

Each out-pocketing arises single, but its distal end, i. e. the blind end, soon begins to be produced in two horns, which then grow out towards the two adjoining interradii, and each horn finally comes to lie between the ectoderm and the hydrocœlic pouch (*cf.* fig. 11 c & d). The horns together with the portion adjoining them are afterwards (in stage F/G) cut off from the rest by the formation of a septum, and give rise to the outer perihæmal ring of authors and the small central portion of the radial perihæmal space stippled in cut 1. In the madreporic interradius exactly similar changes occur, with the only difference that the diverticulum is represented by the

posterior portion of the anterior enterocœl, which about this time begins to assume the form of the axial sinus. The mode



Cut 2.

of formation of the circular enterocœl and the central portion of the perihæmal system is shown in cut 2, where the broken lines represent the union which is secondarily established between the cavities, and completes the circular enterocœl. This definitive separation of the circular enterocœl from the perihæmal system takes place in stage G (fig. 15).

The above statements have been carefully tested and verified in serial sections and I have no doubt of their veracity. It must at the same time be pointed out that the circular enterocœl as well as the central portion of the perihæmal system is a rather composite structure, for, while the larger part arises from the secondary left posterior enterocœl, the part lying in the madreporic interradius is formed from the anterior enterocœl.

With regard to the remaining portion of the radial perihæmal spaces in *Asterina gibbosa*, I find that they arise in exactly the same way as in *Asterias pallida*, i. e. as solid masses of mesenchyme cells placed in pairs on either side of the radial plane, directly on the oral side of the water-vascular tube and between the successive pairs of tube-feet. These masses afterwards acquire lumen and those on one side of the radial plane growing out towards each other fuse, and thus give rise to the definitive condition of the perihæmal system. I have

taken pains to examine closely if the idea of MacBride can not be applied to the entire perihæmal system in this sense, that the cells forming the masses that give rise to the radial perihæmal spaces wander out from the wall of the central portion of the system, which arose, as shown above, from the enterocœl. I have not, however, found any evidence to support this supposition, but on the contrary could trace all the stages from the mass of a few cells to a definite vesicle, such as the one figured in fig. 16. We must therefore conclude that the perihæmal system (*mihi*) arises partly from the enterocœl and partly from the mesenchyme. We may notice that a similar composite origin of evidently homologous structures are known in other groups (*Balanoglossus*, *Ascidia*).

If now we institute a comparison between *Asterias pallida* and *Asterina gibbosa*, we see that there are striking similarities as well as marked differences in their development. The formation of the epigastric enterocœl and of the secondary left posterior enterocœl takes place in exactly similar ways in the two species. The pericœsophageal enterocœl and the stone-canal originate in essentially the same way. As to the "dorsal sac", it arises also from the same cavity in the two species, although it subsequently undergoes different changes, as already described. On the other hand, the circular enterocœl and the central portion of the perihæmal system present markedly different modes of origin in the two forms; the circular enterocœl being formed in *Asterias* from the anterior enterocœl and the entire perihæmal system from the mesenchyme, while in *Asterina* the former is derived both from the secondary left posterior, and the anterior, enterocœl, and the peripheral portion only of the latter from the mesenchyme, the central portion having the same origin as the

circular enterocœl. It must strike one as rather remarkable that such striking differences are to be found in two such nearly allied forms. Further studies will probably throw light on this point and lead to a better unification of the facts known.

September, 1898.

Postscript.

In the discussion on the connection of the axial sinus and the stone-canal contained in my paper on *Asterias* I have quoted the statement of my friend Dr. Cuénot to the effect that, there is no connection between the axial sinus and the neighbouring cavities (p. 271). This statement was extracted from his paper of 1887; but I now find that in his paper of 1891 he distinctly admits the existence of such a connection (p. 542), and I beg here to express my regret for this unfortunate oversight on my part. I must, at the same time, draw attention to the fact that the connection admitted by Dr. Cuénot does not seem to me to be the same as the one demonstrated by me. He admits only the indirect connection that takes place between the stone-canal and the axial sinus by means of the anastomosing madreporic canals that lead into the latter. The connection therefore lies, according to Dr. Cuénot, inside the madreporite, while the one demonstrated by me lies entirely outside the madreporite, as may be inferred from the figures reproduced in my former paper. The connection in question, therefore, takes place, according to my observations, indirectly through the anastomosing madreporic canals and directly through the passage of communication directly under the madreporite, the latter being the primitive

connection existing since larval life. It appears to have been observed by Perrier, as may be seen from a statement in his paper, "Recherches sur l'organisation des étoiles de mer" (Comptes rendus d. l'acad. d. sci. de Paris, 1896, p. 1148). He says, "Si les canalicules de la plaque madréporique ne conduisent que dans le tube hydrophore ou dans son expansion supérieure, le tube lui-même s'ouvre latéralement dans le canal sacciforme....."



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Plate XVIII.

- Fig. 1. Stage B/C. Larva viewed from the right. The broken line shows the outline of the gut. $\times 86$ diam.
- „ 2. The same viewed from the left. $\times 86$ diam.
- „ 3. Stage C/D. Larva viewed from the right. $\times 86$ diam.
- „ 4. The same viewed from the left. $\times 86$ diam.
- „ 5. Stage B/C. Selection from serial transverse sections, each section $=7.5\mu$. There are 70 sections in all; and *a* is section 15, *b* section 20, *c* section 27, *d* section 43, the counting being made from the posterior. $\times 86$ diam.
- „ 6. Stage B/C. Frontal section. $\times 86$ diam.
- „ 7. Stage C/D. Selection from serial transverse sections. There are 90 sections in all; *a* is section 14, *b* 24, *c* 30, *d* 51. $\times 86$ diam.
- „ 8. Stage C/D. Frontal section. $\times 86$ diam.
- „ 9. Stage C/D. From a frontal section; to show the formation of the mesentery leading to the formation of the epigastric enterocœl. $\times 390$ diam.
- „ 10. Stage D. Selection from serial transverse sections. There are 86 sections in all; *a* is section 14, *b* 20, *c* 31, *d* 43, *e* 51. $\times 86$ diam.
- „ 11. Stage E. Selection from serial transverse sections. There are 96 sections in all; *a* is section 12, *b* 17, *c* 38, *d* 43, *e* 47.
- „ 12. Stage E. Selection from serial frontal sections. There are 70 sections in all, the preoral lobe being left out; *a* is section 19, *b* 34, *c* 46, *d* 53, *e* 58. $\times 86$ diam.
- „ 13. Stage E/F. Selection from serial frontal sections. There are 64 sections in all, the preoral lobe being left out; *a* is section 14, *b* 21, *c* 26, *d* 30, *e* 34, *f* 42, *g* 46. $\times 86$ diam.
- „ 14. Stage F. Selection from serial frontal sections. There are 79 sections in all; *a* is section 16, *b* 21, *c* 23, *d* 25, *e* 29, *f* 32, *g* 40, *h* 43, *i* 47, *k* 54, *l* 56. $\times 86$ diam.
- „ 15. Stage G. Selection from serial frontal sections. There are 88 sections in all; *a* is section 30, *b* 43, *c* 51, *d* 61, *e* 68, *f* 73. $\times 86$ diam.
- „ 16. Stage F. From a frontal section. The circular enterocœl and the central portion of the perihæmal system still forms a single cavity (*ent. cir.*); the mesenchymatous portion of the perihæmal system is represented by a closed vesicle (*perihæm.*). $\times 390$ diam.
- „ 17. Stage C/D. Portion of four successive transverse sections, to show the origin of the "dorsal sac." $\times 146$ diam.

Abbreviations.

<i>ant.</i>Anterior enterocœl.	<i>p. s.</i>Left posterior enterocœl.
<i>can. aq.</i>Pore-canal.	<i>p'. s'.</i>Secondary left posterior enterocœl.
<i>can. aren.</i>Stone-canal.	<i>perioes.</i>Perioesophageal enterocœl.
<i>ent.</i>Enteron.	<i>perihæm.</i>Perihæmal space.
<i>ent. cir.</i>Circular enterocœl.	<i>sac. dor.</i>"Dorsal sac."
<i>epigas.</i>Epigastric enterocœl.	<i>sin. ax.</i>Axial sinus.
<i>hydr.</i>Hydrocœl.	<i>vas. aq.</i>Water-vascular canal.
<i>p. d.</i>Right posterior enterocœl.	<i>vas. aq. cir.</i>Circular water-vascular canal.

The Arabic numerals denote the primary hydrocœl pouches.

