

*On the Origin of the Ascidian Mouth.*

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A year ago I undertook to determine the origin of the test-bearing and testless parts of the epithelial lining of the siphons of Ascidians. My material consisted of adult *Dendrodoa* (*Styelopsis*), *Cæsiira* [*Molgula*], and *Clavelina*, each containing embryos of all stages. These were studied in serial sections.

In the case of the oral siphon, I reached the unexpected result that a large part of the epithelium of the wall of the oral cavity is derived from the primitive neural tube of the embryo. It can readily be understood that the connecting of definite parts of the epithelium of the adult with definite parts of the germ layers of the embryo cannot be done with any great certainty. For several reasons I conclude that the outer limit of the "neural" epithelium of the oral siphon is the margin of the test-bearing epithelium (indicated usually by a distinct ridge or velum), and that the inner limit is the peripharyngeal band or ridge.

The mouth of the Ascidian corresponds strictly with the neuropore of the embryo. The neuropore closes rather early, but re-opens during or after metamorphosis to form the connection between the test-bearing and testless parts of the siphon.

In the three genera investigated I have been unable to find any stage in which the neural tube is separated from the ectoderm at its anterior end, as maintained by Kupffer, Kowalevsky, and Seeliger. I have examined numerous stages (both a year ago and recently), from that of the open medullary tube to the escaped larva, and in all the neural tube is connected with the ectoderm.

The accounts that have been given of the manner in which the stomodæum, the hypophysial canal, and the pharynx become connected are more or less conflicting.

Kupffer (1870) failed to find a neuropore at any stage. He describes the fusion of a stomodæal invagination, with the pharynx to form the mouth.

Kowalevsky (1871) describes the early closure of the neuropore, and derives the mouth from an invagination of the ectoderm, which connects almost simultaneously with the sensory vesicle of the neural tube and with the pharynx.

Willey (1893) describes the early closure of the neuropore and the formation of the mouth by the fusion of the stomodæal invagination with the pharynx. Shortly (in *Clavelina*) or some time (in *Ciona*) after the formation of the mouth, the hypophysial canal connects up with the base of the stomodæum. He does not appear to be perfectly certain of the way in which this latter connection is made.

Seeliger (1904) considers that the neuropore closes early and that the neural tube separates from the ectoderm. Later, the hypophysial canal separates from the sensory vesicle, and grows forward to unite with either the stomodæum, the pharynx, or the junction of the two. But he does not seem to think that the details have been satisfactorily established.

As Willey considered that the point where the hypophysial canal broke through into the stomodæum corresponded with the previously closed neuropore, my conclusions are somewhat in harmony with his. I have not had the opportunity of studying the origin of the mouth in *Ciona* or in a Phallusiid, and possibly in these the neural tube separates from the ectoderm. But in *Clavelina*, which was studied by Willey and Seeliger, I do not believe that the separation occurs. Willey did not consider this point. Seeliger stated that separation occurred, but I am inclined to think that he did not actually investigate it in *Clavelina*. He states, however, that he examined larvæ in which the opening of the hypophysial canal was present, but in which the mouth was not yet broken through.

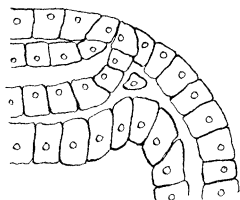
I have been unable to find a stage in which the canal is connected with the pharynx and not with the exterior. If an actual lumen be implied, Seeliger's statements are confirmed by my findings. But the essential thing is the arrangement of the cells to form a tube, whether closed or open.

When observations are made on entire embryos, the connection of the neural tube with the ectoderm is easily overlooked. Also the fixation of the material or the subsequent handling might break the connection. My material was fixed with Zenker's fluid, and the embryos remained during preservation, embedding and cutting *in situ* inside the adult. This treatment prevented any separation of the parts of the embryo, but made the examination of the sections somewhat difficult.

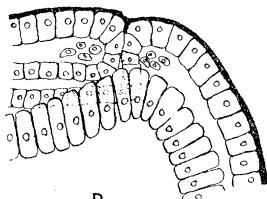
In the early stages of development the neural tube is widely open in front, but at the time when the tail grows out, the neural tube is closing. The margins of the neuropore come together and from the outside little if any indication remains of the position of the neuropore. In *Clavelina* there is usually a slight depression, more or less filled by testa cells. A section of this stage (fig. 1A) shows the neural tube still connected with the ectoderm, but a definite lumen cannot be traced to the surface of the ectoderm. The

position of the original lumen is always indicated by the way in which the cells are arranged. The anterior end of the tube is bent upward to a slight extent (when the neuropore is open the anterior end is straight).

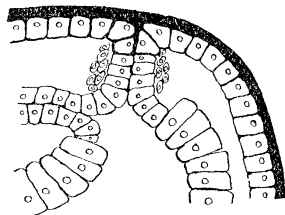
When the anterior end of the neural tube separates into a dilated sensory



A



B



C

FIG. 1.—Diagrammatic sagittal sections through antero-dorsal part of Ascidian embryos of three different ages, showing transformation of neural tube into wall of oral siphon.

vesicle (on the right side) and a narrow hypophysial canal (on the left side), it is the latter that retains a connection with the ectoderm. In this stage the anterior part of the canal is bent into the form of a right angle (fig. 1B) and the ventral part of the angle has sunk into the endoderm. The endodermal cells are large and columnar. They separate to make room for the canal. Usually a distinct lumen can be seen in the horizontal part of the canal but not in the vertical part. Grouped around the latter are mesodermal cells forming a characteristic ring. The ectoderm at the neuropore has commenced to invaginate and as the test is appearing the invagination is easily distinguished.

In the next stage (fig. 1C) the hypophysial canal has opened into the pharynx at the angle. The margins of the opening in the wall of the canal have become connected with the margins of the opening made in the endoderm by the sinking of the canal. This causes a great increase in the lumen of the canal at the angle, where this connection occurs. The vertical limb (which we may now call the oral siphon) is still without a lumen and the horizontal limb has little or no lumen. The vertical limb or oral siphon has become longer, partly from a lengthening of the neural part and partly from a further invagination of ectoderm. The layer of test has increased in thickness.

The fully-developed larva shows only unimportant advances on this condition. The oro-pharyngeal opening becomes larger, the oral siphon longer and the layer of test thicker. The oral siphon remains closed till after the metamorphosis.

The above description and the figures are based upon the condition in *Dendrodoa*, but the process is essentially the same in *Clavelina* and *Cæsira*.

In the latter form the extent of the neural tube material is more easily seen than in either of the other forms, since the cells of the neural tube contain much less yolk than those of the ectoderm and endoderm.

There is great difficulty in tracing the further fate of the neural, ectodermal and endodermal cells respectively of the oral region. I believe, however, that the following conclusions are warranted.

At the time when the ectodermal cells begin to invaginate at the position of the old neuropore, test has begun to form on the surface of the ectoderm. The cells invaginated have test on what are or will be their free surfaces. Doubtless they continue the formation of test. If so, the ectodermal part of the adult will consist of the test-bearing epithelium of the siphon, the so-called "reflected tunic." The hypophyseal canal of the late embryo and larva opens near the margin of the endoderm (fig. 1c). If this position is retained, the junction of the neural and endodermal regions of the adult will be immediately behind the dorsal tubercle. In this position we have the peripharyngeal ciliated bands. These connect ventrally with the right and left lips of the endostyle. The peripharyngeal bands have every appearance of being a dorsal continuation of the endostyle, split into right and left halves by the sinking down of the neural tube. The subsequent fusion of the latter with the margins of the opening thus formed in the pharynx causes the two halves of the split endostyle to form a circular band at the anterior extremity of the pharynx.

The parts derived from the three embryonic layers will then be,

Ectodermal.....	Test-bearing epithelium or "reflected tunic."
Neural .....	Pretentacular zone.
	Tentacular ridge and tentacles.
	Prebranchial zone and dorsal tubercle.
Endodermal ...	Peripharyngeal bands and parts behind.

This is illustrated in fig. 2, a diagrammatic longitudinal section through

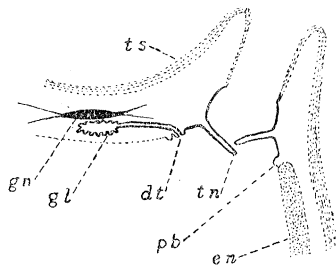


FIG. 2.—Diagrammatic sagittal section through oral region of adult Ascidian. *dt*, dorsal tubercle; *en*, endostyle; *gl*, neural gland; *gn*, ganglion; *pb*, peripharyngeal band; *tn*, tentacle; *ts*, test.

the oral region of an Ascidian, in which all parts, except those of neural origin, are represented by dotted lines.

The three genera that have been the subject of this investigation belong to three different families, and one of them (*Clavelina*) is almost at the opposite extreme of the Ascidian series from the other two. I presume, therefore, to think that this origin of the mouth is characteristic of all or of most Ascidians, and that any exceptions will show only modifications of this. It affords a satisfactory explanation of the opening of the neural gland or hypophysis into the oral siphon.

As to the general significance of this developmental process, I think that comparisons between this process and that to be found in Cephalochordates and in Vertebrates should be used with much caution. I believe that the majority of the homologous organs of the three groups have been evolved independently, and are so remarkably alike because they have been derived from an original similar condition in the groups, this condition being the constitution of the germ plasm rather than the presence of these organs.

With this reservation, I make the following homologies (most of which have been made previously), with the organs in order from behind forwards and downwards.

Tunicate.	Cephalochordate.	Vertebrate.
Ganglion .....	Ant. end of nerve cord...	Main part of brain.
Sensory vesicle { Eye .....	Eye-spot .....	Pineal eye and retina of lateral eyes.
Otolith ...	...	Auditory vesicles.
Neural gland and duct .....	Olfactory pit .....	Hypophysis and nasal cavities.
Neural part of oral siphon...	Stomodæum .....	Stomodæum.

The order is faulty only in the case of the auditory vesicles of Vertebrates. They occur laterally and behind the eyes.

The three groups form a series as to the connection of the different organs with each other and as to the times at which they invaginate from the ectoderm. In the Tunicate they are all connected together and invaginate very early to form the neural tube. In the Cephalochordate the first two and the fourth (the third is absent) are connected. The first two form part of the neural tube, whereas the fourth invaginates later but at the neuropore. The fifth (stomodæum) is separate from the others. In the Vertebrate only the first two are connected together and form part of the neural tube. The others are more or less separate and invaginate later.

These structures are all median and unpaired, except the three sense organs of the Vertebrate, which are usually paired.

These groups also form a series with reference to the amount of rotation of these organs around the anterior end of the body. In the Tunicate they are all dorsal. In the Cephalochordate they are all dorsal except the last. In the Vertebrate the last two are ordinarily ventral and exceptionally nearly all of them may come to the ventral side.

It may be noticed that the condition in the Tunicate strongly supports the view that the neural tube was originally not nervous but a part of the digestive system.

I hope shortly to publish a more extended account of the later embryonic development of the Ascidian. I wish to express here my appreciation of the splendid facilities for research and for the collection of material which were placed at my disposal by the Biological Board of Canada, both at the Atlantic Station, St. Andrews, New Brunswick, and at the Pacific Station, Departure Bay, British Columbia. My material was obtained at these stations.

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