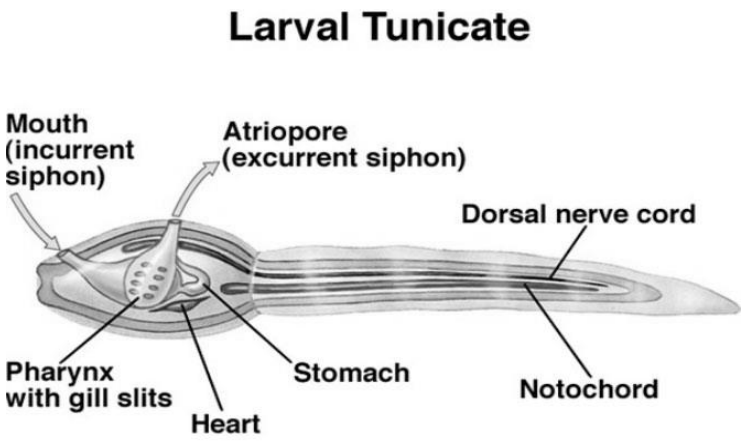
Retrogressive metamorphosis in ascidians.

Subphylum Urochordata: Class 1. Ascidiacea: Examples- *Herdmania*, *Ciona* and *Molgula*.

Class 2. Thaliacea: Examples- *Salpa, Doliolum, Pyrosoma*.

Class 3. Larvacea or Appendicularia: Examples- *Oikopleura*, *Appendicularia*.

The type of metamorphosis which shows degenerative or retrogressive changes from larva to adult is called retrogressive metamorphosis.



Development of ***Herdmania*** has been described by Sebastian (1953) and Das (1957).

Development is indirect involving a free swimming tadpole larva.

Herdmania (a tunicate) is the most appropriate one of the best examples of retrogressive metamorphosis in which the highly developed tadpole larva undergoes retrogressive changes to become the most degenerated and sedentary adult.

The phenomenon of retrogressive metamorphosis is peculiar to urochordates and provides a clue to their chordate nature.

- **Gametes:**

- Spermatozoa: The sperm of Herdmania is flagellated and about 4 μm in length. Each sperm has an anterior head bearing a middle nucleus and capped with a beak-like acrosome, a middle piece with neck and a very long tail. According to Das (1936), sperms of Herdmania are polymorphic, i.e., three types of sperms having *acrosome* shorter, equal or longer than the head.

- Ova: The ova or unfertilised eggs are microlecithal having distinct animal and vegetal poles. Mature ovum is about 0.3 mm in diameter containing an eccentric nucleus with a prominent nucleolus. The egg is surrounded by an inner *vitelline membrane* secreted by the ovum which is closely applied to it, and outer to it are two *chorion membranes* inner and outer secreted by follicular cells of the ovary.

The space between inner chorion and vitelline membrane is the *perivitelline space* filled with *perivitelline fluid*. The perivitelline space contains a large number of small-sized inner follicle cells or test cells, some of which float in the fluid and most of them are attached to vitelline membrane.

These cells *nourish* the ovum and *secrete* a chorion digesting enzyme to facilitate hatching.

Follicle cells are also found attached to the outer surface of chorion membrane. These cells *keep the ovum floating in outer perivitelline fluid*.

Maturation of ovum occurs in sea water.

Fertilisation:

Fertilisation is external and cross-fertilisation. Spermatozoon penetrates the ovum nearer to vegetal pole. Polyspermy is not allowed by the chorion layers.

After fertilisation, various cytoplasmic regions are rearranged by a process known as *ooplasmic segregation*, i.e., yellow and clear cytoplasm segregates in the lower hemisphere with a cap of clear cytoplasm over it and gray yolk-laden endoplasm occupies the upper two-thirds of the egg.

Larval Development:

The blastopore closes and develops a rudiment of tail. The embryo elongates and forms a tailed larva. The presumptive notochordatal cells separated from the roof of the archenteron, occupy the central core of larval tail.

Archenteron produces presumptive mesoderm as solid bands and not as hollow coelomic sacs as in *Balanoglossus* or *Branchiostoma*. About 8 hours after fertilisation, the chorions rupture, probably dissolved by an enzyme secreted by the inner follicle cells, and the fully formed larva hatches out to become free-swimming.

Tadpole Larva:

- The fully developed ascidian tadpole is minute, about 1.5 mm long and motile. Its body is covered by a thin tunic or test secreted by the ectoderm. It has an anterior short oval head or trunk and a posterior long tail.

- **1. Head or Trunk:**

- At the anterior end of the trunk are three adhesive papillae made of ectodermal cells. The nervous system consists of an anterior enlarged hollow sense vesicle or brain vesicle. The sense vesicle is continued posteriorly into a solid, thick mass of nerve cells, the visceral ganglion, it is continued posteriorly into a nerve cord which is hollow and continued into the tail. It lies mid-dorsally.

- The sense vesicle contains a pigmented statocyst (otocyst) and two pigmented unequal ocelli or simple eyes as sense organs. The alimentary canal begins from antero-dorsal mouth leading into narrow branchial siphon, a large sac-like pharynx, short oesophagus, stomach, intestine and a small rectum. The anus opens into the left side of atrium. The pharynx has a ventral endostyle and also a pair of large gill-slits or stigmata.

- Each gill-slit finally splits to form six stigmata on either lateral side An atrial cavity is formed around the pharynx laterally and dorsally and opens to the exterior by an atrial aperture lying dorsally. The heart and pericardium is located postero-ventrally in between pharynx and stomach. Mesenchyme cells found scattered all over the body beneath the ectoderm and in a mass in the posterior region of trunk.

- **2. Tail:**

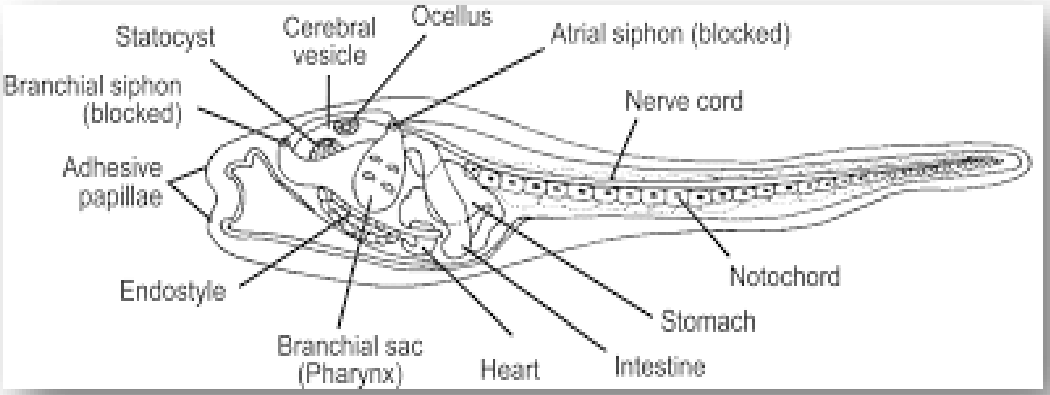
- It is a powerful locomotory organ being laterally compressed and pointed terminally. Tail is about 0.9 mm long and is fringed with a vertical continuous tail fin formed by the test and marked with oblique striations looking like fin rays. It contains a dorsal tubular nerve cord, a notochord beneath the nerve cord and lateral muscle bands. The anterior end of notochord slightly projects into the trunk region.

- The ascidian tadpole larva is unquestionably a true chordate, having a dorsal hollow nerve tube, a notochord in the tail and paired gill-slits in the pharynx and after about three hours of active, non-feeding and free-swimming existence, it sinks to the bottom.

- **Habits and Habitat of Larva:**

- Just after hatching, the larva is photopositive and *geonegative*. It cannot feed because its mouth is still closed by test. After a short active free-swimming existence lasting about 3 to 4 hours, the larva becomes *geopositive, photonegative* and *sluggish.*

- It sinks to the bottom, attaches itself upside down to a suitable hard substratum by adhesive papillae and undergoes rapid degeneration or retrogressive metamorphosis to attain adulthood. According to Berril (1955) the selection of a suitable habitat is essential as the larva may not survive on any other habitat or may get suffocated by the bottom mud and detritus.



- **Retrogressive Metamorphosis:**
- Metamorphosis Gr., meta = after + morphe = form + osis = state) is the shape change in form during post-embryonic development and in many cases, signals a dramatic change in habitat of the animal such as pelagic to benthic existence.
- Metamorphosis of the ascidian larva **is unique and begins almost explosively.** It involves *transformation of* an active non-feeding, pelagic, lecithotrophic (i.e., that feeds on its own yolk reserves) and tailed larva having many advanced features such as axial notochord, dorsal neural tube and special sense organs, *into* an inert, sedentary or sessile, simple (primitive) and plankotrophic filter feeding adult with only a phaynx with stigmata and endostyle, *both indicating the chordate features of adult ascidian.*
- This type of metamorphosis which shows degenerative or retrogressive changes from larva to adult is called retrogressive metamorphosis.
- **It involves the following three types of changes:**
- (i) Retrogressive,
- (ii) Progressive and
- (iii) Molecular changes.

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- **(i) Retrogressive Changes:**
- **These changes involve destruction and disappearance of some of the larval structures as follows:**
- a. Long tail of larva with caudal fin shortens and finally *disappears*.

- b. Caudal muscles, nerve cord and notochord *disappear* as they break down and are consumed by phagocytes.
- c. Larval sense organs (the ocellus and the otolith) are *lost* and sensory vesicle is *transformed into* an adult cerebral ganglion.
- d. Adhesive papillae *disappear* completely.
- e. Anterior region between point of attachment (adhesive papillae) and mouth shows *rapid growth*, while original dorsal side with atriopore *stops growth*. This causes *shifting of mouth through 90°*. Therefore, the final branchial and atrial apertures in the adult represent the *original anterior* and *dorsal sides of the larva*.
- **(ii) Progressive Changes:**
- **Some larval structures necessary for survival become more elaborated and specialized in each adult, such as:**
- **a**. Due to loss of tail, the trunk becomes pear-shaped and four larger ectodermal ampullae grow out of its four corners. These ampullae firmly anchor the metamorphosing tadpole to the substratum and also serve for respiration as a blood-like fluid keeps circulating through them. Soon two more small ectodermal ampullae appear dorso-laterally.
- **b**. Anterior region between point of attachment (adhesive papillae) and mouth exhibits rapid growth, while original dorsal side with atriopore stops growth. This causes shifting of mouth through 90°. The body too rotates so that general form of the adult sessile organism is assumed.
- **c**. Adult neural glands and nerve or cerebral ganglion are formed by the neural tube and trunk ganglion comes to lie mid-dorsally between mouth and atriopore. The trunk ganglion itself persists as visceral nerve.
- **d**. With the absorption of its test covering, the mouth becomes functional and filter-mode of feeding by incoming ciliary water currents.
- **e**. Pharynx greatly enlarges, develops blood vessels and stigmata multiply rapidly, forming the branchial sac.
- **f.** Stomach enlarges, intestine elongates and gets curved and liver develops.
- **g.** Atrial cavity becomes more extensive.
- **h.** Circulatory system with heart and pericardium develops.
- **i**. Gonads and gonoducts develop from larval mesodermal cells.

- **j**. Test or tunic spreads to cover entire animal, becomes thick, tough and vascular and attaches the animal by forming a foot if necessary.
- Thus, foregoing metamorphic changes mark the beginning of a sedentary, actively feeding, sexual adult life which soon starts producing gametes, first ova and later sperms.
- **(iii) Molecular Changes:** (Manket and Cowden, 1965) studied the molecular changes which take place during metamorphosis. They studied the metabolism of protein and nucleic acid and pointed out that some protein synthesis occurs throughout the development but with the outset of metamorphosis; extensive degradation of proteins begins followed by rapid synthesis of new proteins.
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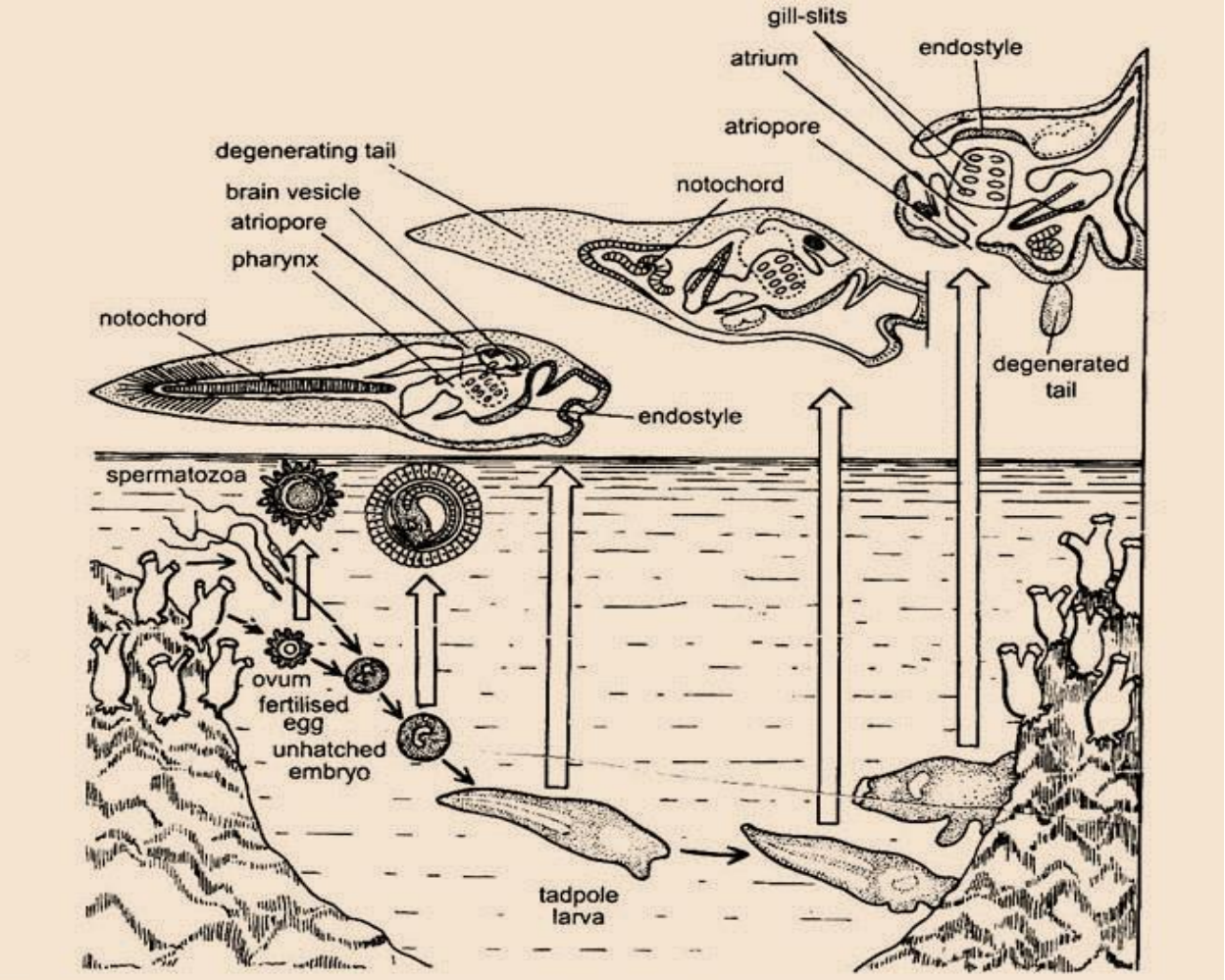

Fig. 35.4. Life cycle of a simple ascidian, showing retrogressive metamorphosis of tadpole larva, later its attachment with the solid substratum.

- **Embryological Significance of Ascidian Tadpole:**
- **The presence of a tadpole larva in the life history of *Herdmania* and other ascidians is significant in the following ways:**
- **a. Taxonomic Significance:**
- The tadpole larva possesses true chordate characters such as notochord and dorsal tubular nerve cord, which are lacking in the adult. Thus, the ascidian larva provides the clue for including the ascidian under the phylum Chordata. Without tadpole larva, the true nature and taxonomic position of degenerate sedentary adult ascidians would have remained uncertain.
- **b. Phylogenetic Significance:**
- On the basis of recapitulation, the ascidian larva possessing the chordate features is considered as the relic of the free-swimming ancestral vertebrates.
- **c. Dispersal:**
- The adult ascidians being sedentary, the free-swimming habit of the larva provides the only means of dispersal of the species. It also provides chances of selecting better sites regarding food and protection, thus, ensuring survival of the race.
- **d. Embryological Significance:**
- Ascidians provide best example of mosaic eggs with a well- organized, pre-patterned and well differentiated ooplasm and highly determinate type of development. Moreover, ascidians are the only chordates exhibiting true retrogressive metamorphosis.
- The egg cortex in case of ascidians is the site of morphogenetic patterning related to polar, bilateral and general organization of developing egg. Besides this, cleavage in ascidians tends to segregate cytoplasmic territories, having different biological, histochemical and biochemical properties.
- Cloney (1982) classified the ascidian tadpole structures into 3 categories and the features are represented in Table

Table 14 : Classification of the Ascidian tadpole structures

Transitory larval organs	Prospective juvenile organs	Larval juvenile organs and tissues
i. Presence of notochord	i. Presence of branchial and atrial siphons	i. Presence of inner compartment of tunic
ii. Presence of visceral ganglion and nerves	ii. Presence of branchial basket	ii. Presence of mesenchyma
iii. Presence of dorsal tubular nerve cord	iii. Presence of oesophagus, stomach and intestine	iii. Presence of blood cells and heart (found in some species).
iv. Presence of papillae	iv. Presence of heart (found in some species) and epicardium	
v. Presence of outer cuticular layer of tunic	v. Presence of cerebral ganglion and neural gland.	
vi. Presence of sensory vesicle and sensory organs		
vii. Presence of tail muscles.		

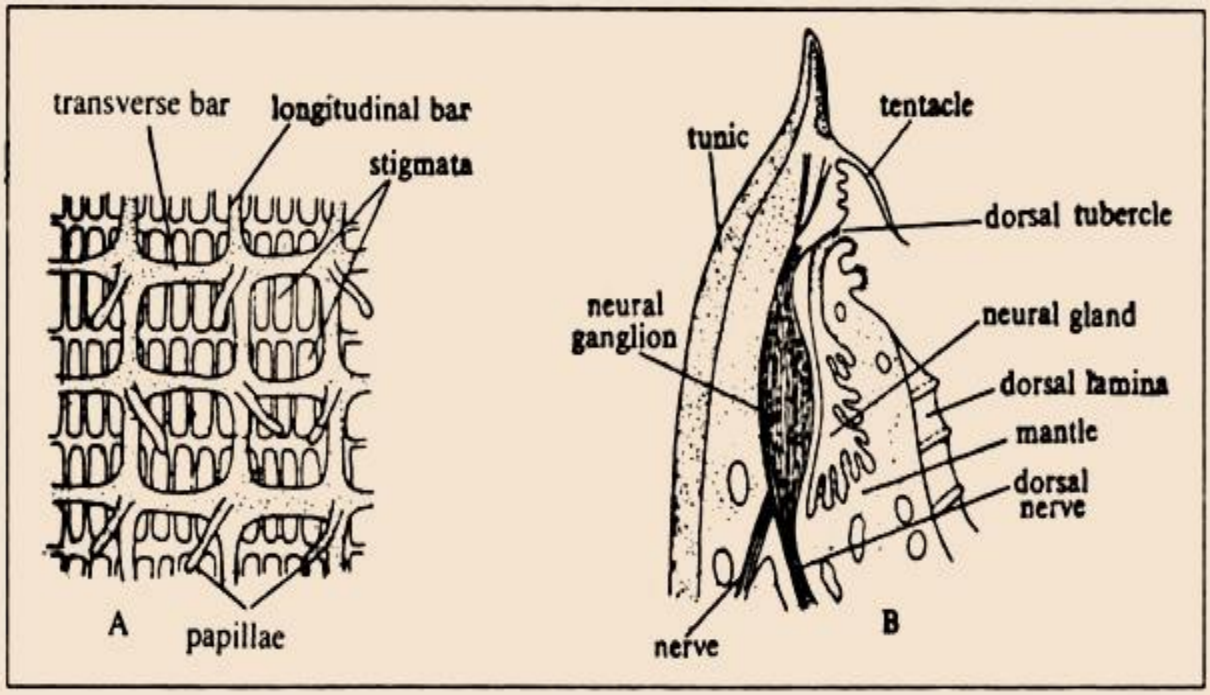

Fig. 3.3 : A. A greatly enlarged view of a portion of the pharyngeal wall of *Ascidia*. B. A sagittal sectional view of the anterodorsal part of the body wall to show the relationship between the neural ganglion and neural gland in *Ascidia*.

- **Experimental analysis for the metamorphosis of ascidian tadpole:**

- Some physical and chemical factors are concerned with the metamorphosis of ascidian tadpole. Herdman regards phagocytosis as the primary cause of larval tissue destruction while Berril (1929) advocates that the relative tissue starvation is responsible for larval tissue destruction. Grave (1935) believes that a metabolic product of swimming activity is essential for metamorphosis.

- **Grave (1935) concludes that normal metamorphosis of tadpole is conditioned by two factors:**

- (i) Ageing of the larva after the liberation, and

- (ii) Swimming activity.

- Glaser and Angslow (1949) attempted to assign the controlling role of copper during metamorphosis, released in the tadpole tissues. Some other factors like metabolic ions, iodine, low concentrations of vital dyes, some amino acids, thyroxine extracts, and exposure to hypotonic sea water, dimethyl sulpoxide, acetylcholine and favorable lightening condition may be recorded to accelerate the metamorphosis.

- **Molecular analysis of Ascidian metamorphosis:**

- Scientists have succeeded to isolate some genes which are related to ascidian metamorphosis. *Five novel genes* — manx, lynx, cymric, p^{58} and bobcat have been isolated from the ascidian species. Lynx and Cymric are maternal and experiments are underway to see their roles in the development.

- Manx, p^{58} and bobcat are expressed both maternally and zygotically in the tailed species, and antisense experiments suggest a role in specifying the body plan during development. Bobcat is also seen to be expressed in the neural tube of chordate embryos.

- In addition, 132 different protein coding sequences have been isolated, of which 65 of these transcripts show significant matches to Gen Bank proteins. Some of these genes have putative functions relevant to metamorphic events, related to the differentiation of muscles, blood cells, heart tissue and adult nervous systems.

- One set of genes that are activated at metamorphosis are the innate immunity genes. These genes are related to the *expression of immune system which is critical for remodeling the body plan during metamorphosis.* In addition, this immune system may be necessary for phagocytosis and restructuring of larval tissues.

- **Zoological Importance of Tadpole Larva in Ascidia:**

- The presence of tadpole larva in the life- history of Ascidia is very significant. From the taxonomical standpoint, the tadpole larva helps us to include the Ascidians under the Phylum Chordata, otherwise their inclusion under the phylum would have been questionable.

- The existence of tadpole in their life-cycle appears to be either a case of recapitulation of the past racial history or it must be the birthright of a chordate. However, the tadpole larva can be regarded as ***a relic of the ancestral free-swimming chordate.***

- Two contradictory views exist on this particular issue. The first view holds that the existence of tadpole larva is an interpolation in the life-history of Ascidia which — by the suppression of metamorphosis and further evolution — might have given origin to the ancestral chordate. The other view holds that the existence of tadpole larva is not a case of interpolation.

- It has evolved within the group to meet certain Ascidian needs. The tadpole larva holds the key position, from which the vertebrates have emerged out in space and time. However, in recent days, the tadpole larvae are not regarded as the ancestors of vertebrates.
