STRUCTURE, TADPOLE AND BUD FORMATION IN THE ASCIDIAN *ARCHIDISTOMA*

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(Text-figs. 1–6)

Archidistoma is a genus created for a species discovered and described briefly by Garstang in 1891 as *A. aggregatum.* It has been found at different times only in the Plymouth region, the original locality, attached to rock surfaces in a few feet of water. Recently, however, it has been found near Cape Hatteras on the eastern American coast (Van Name, 1945), suggesting either harbour to harbour transportation by ship bottom, or that its inconspicuous appearance has obscured a wide distribution.

This form is of considerable interest as it represents a type less specialized in zooid structure, nature of tadpole, and method of budding than any merosomatous ascidian except the diazonids. Diazonids are oviparous and have the simpler development and tadpole associated with small eggs, although there is a more elaborate branchial sac correlated with the relatively large size of the mature zooid.

Colony

The colony consists of a brownish tough matrix forming a thin encrustation on stones and shells, with individual zooids (a few millimetres long), partly embedded in the test but for the most part extending freely in clumps usually of three or four. Sand grains and round particles adhere to or become buried within the test, making this form the most inconspicuous of ascidians.

Structure of Zooid

The zooid is divisible into a thorax and a long slender abdomen (Fig. 1). There is no postabdomen, nor is there hypertrophy of the ventral test vessels, thereby segregating *Archidistoma* as a type from that of the synoicids (polyclinids) and the clavelinids respectively. The relatively great length of the oesophageal region of the abdomen also marks it off from the didemnids and *Distaplia*.

Both branchial and atrial siphons are well developed and are independent, the atrial siphon having six unmodified lobes like those of the branchial siphon. This is correlated with the absence of true zooid systems and is undoubtedly a primitive feature.

The thorax is short and wide, as is the contained endostyle. Tentacles are simple and about thirty in number. There are three rows of straight stigmata,

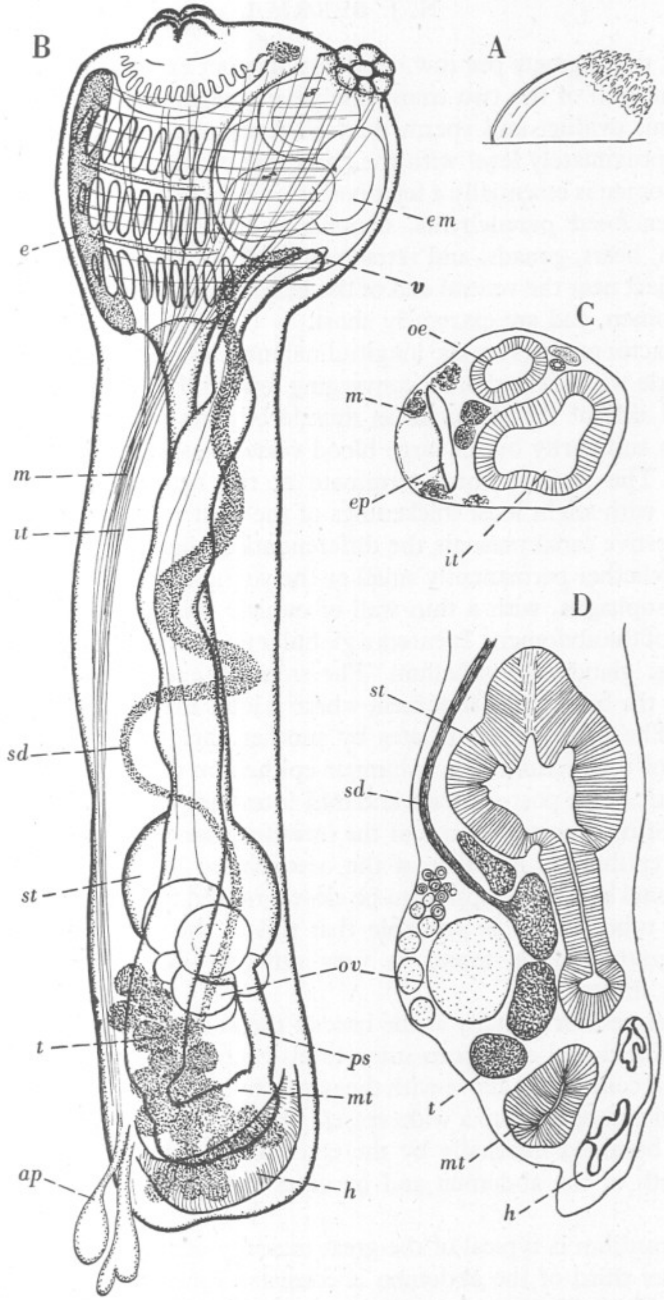

Fig. 1. General structure of *Archidistoma aggregatum*. A, colony growing on bivalve shell, about natural size; B, mature zooid from left side showing organization of thorax and abdomen; C, cross-section through upper part of abdomen showing epicardium; D, longitudinal section of lower part of abdomen showing relationship of gonads and heart to digestive tube. *ap*, ampulla; *e*, endostyle; *em*, embryos in atrial cavity; *ep*, epicardium; *h*, heart; *it*, intestine; *m*, retractor muscle; *mt*, mid-intestine; *oe*, oesophagus; *ov*, ovary; *ps*, post-stomach; *sd*, sperm duct; *st*, stomach; *t*, testicular follicle; *v*, distal ends of oviduct, sperm duct and rectum opening together into base of atrial chamber.

with about ten stigmata per row. A dorsal languet extends into the branchial cavity from each of the two transverse vessels separating the stigmata rows. The rectum, oviduct and sperm duct all terminate well short of the atrial siphon, approximately level with the basal row of stigmata (Fig. 1B).

The abdomen is essentially a long posterior extension of the epidermal body wall and enclosed parenchyma, containing the U-shaped digestive canal, epicardium, heart, gonads, and retractor muscles. The ventral ampullary test vessels project near the ventral end of the heart close to the posterior extremity of the abdomen, and are extremely short.

The retractor muscles are the longitudinal muscles spread somewhat fanwise in the mantle wall of the thorax, converging toward the base and passing down the ventral side of the abdomen as muscle bundles separated partly by the epicardium and partly by the large blood vessel passing from the heart to the endostyle. The muscle fibres terminate at the extreme abdominal tip in association with slight local thickenings of the epidermis.

The digestive canal presents the differentiation characteristic of most small ascidians, whether permanently small or the young of larger forms. A narrow straight oesophagus, with a thin wall of cubical epithelium, descends to the lower part of the abdomen. It enters a globular thick-walled stomach composed of columnar glandular epithelium. The stomach extends as a narrow post-stomach to the base of the abdomen, where it joins the almost horizontal mid-intestine. The latter communicates by another narrow constriction with the lower end of the rectum. The columnar epithelium of the stomach is equally characteristic of the post-stomach and mid-intestine. The rectum, which forms the whole of the ascending limb of the intestine, has a thinner wall consisting of cubical epithelium like that of the oesophagus. The various divisions of digestive canal as a whole appear to be closely related to the locations within the abdominal tube, and it is probable that this is the primitive arrangement, similar digestive canals found in very different locations having become secondarily shifted.

The heart lies horizontally at the base of the abdomen, just below the mid-intestine. The dorsal end opens into vessels branching over the region of the stomach and connecting above with the parenchymal blood channels in general. The ventral end opens into a wide vessel lying between two bundles of muscle fibres and bounded internally by the epicardium. This vessel passes up the whole length of the abdomen and becomes the subendostylar vessel in the thorax.

The epicardium is typical of the great majority of merosomatous ascidians. In the lower third of the abdomen it consists of a single cavity lined by an extremely thin epithelium, lying between the digestive canal and the ventral aortic blood vessel. It ends blindly posteriorly, but anteriorly above the level of the stomach, the right and left divisions, detectable even when fused, become separate from one another and extend as a pair of sacs to the base of the thorax.

There they end blindly as a pair of horns immediately beneath the floor of the pharynx (from which they were originally derived).

The gonads also occupy the position characteristic of most enterogonid ascidians, namely, in the general vicinity of the intestinal loop between the stomach and the mid-intestine. The small spherical testicular follicles extend farther posteriorly to the level of the heart, that is, as far as they can go, and do not extend quite as far anteriorly as the ovary which reaches the lower part of the stomach. To a significant extent, the ovary, or at least the relatively large ova, are surrounded by the follicles of the testis. The oviduct follows the rectum to open alongside the anus into the base of the atrial cavity. The sperm duct does the same except that it takes a somewhat more tortuous course. The arrangement both of the gonads and their ducts is undoubtedly primitive and common to the majority of ascidians.

Sexual Reproduction

Eggs pass up the oviduct and undergo their development as far as the active tadpole stage within the atrial cavity, taking up so much space there that the branchial sac may become pushed very much to one side. Fertilization probably occurs within the oviduct, although this is not known for certain. Eggs are of 0·23 mm. diameter and are extremely yolky, closely resembling those of the synoicids and likewise undergoing epibolic gastrulation.

Considerable increase in size occurs during the development of the embryo, although at the time of liberation of the tadpole a mass of relatively large yolky endodermal cells still remain more or less unutilized, lying between the pharynx and digestive canal, alongside the epicardium. The paired peribranchial ectodermal invaginations fuse to form the single median atrial siphon while yet in the embryonic phase.

The tadpole larvae (Fig. 2) have a short free-swimming period, on an average of about 2 hr. Their organization is interesting in several ways, being a generalized type compared with those of the synoicids, didemnids, clavelinids and holozoans.

The larval structures are rather large relative to the size of the trunk. The tail is long, the sensory vesicle large, and the permanent organs occupy practically all the remaining space, as though there had been a restriction of the surface area of the epidermis. The sense organs are typical, though individually relatively large, and consist of a unicellular otolith and an ocellus bearing three unicellular lenses (Fig. 3B). The tail, as in all viviparous (large-egged) enterogonid ascidians, has undergone a 90° torsion, so that the tail fin is horizontal and the nerve, no longer dorsal, runs along one side (Fig. 3C). It has the universal number of approximately forty notochord cells, while the muscle cells, in a relatively advanced state of differentiation, are arranged in series as three bands down each side of the notochord. Anteriorly the three adhesive organs project on long slender stalks, in triangular arrangement, each organ consisting of a central adhesive papilla and a marginal epidermal cup or sucker.

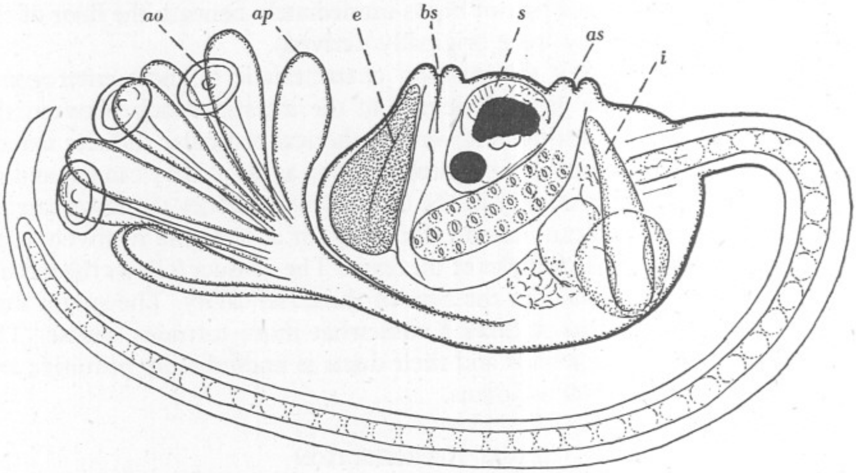

Fig. 2. *Archidistoma aggregatum*. Tadpole larva, with eight ampullae and three adhesive organs anteriorly, a relatively large sensory vesicle, and three rows of perforated stigmata on each side. *ao*, adhesive organ; *ap*, ampulla; *as*, atrial siphon; *bs*, branchial siphon; *e*, endostyle; *i*, intestine; *s*, sensory vesicle.

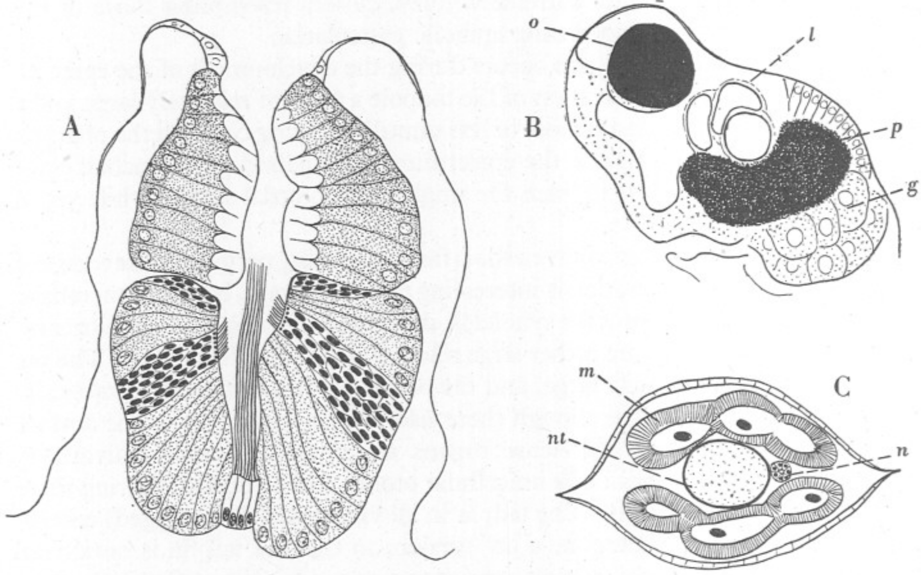

Fig. 3. *Archidistoma aggregatum*. A, section through endostyle of adult, showing lateral bands of large glandular cells alternating with bands of small ciliated cells, and a median ventral band with very long flagella; B, sensory vesicle of tadpole with unicellular otolith, and typical three-lens ocellus; C, section through tail, showing notochord, neural tube, and lateral muscle bands of three cell-rows (highly differentiated). *g*, ganglion cells; *l*, lens cells; *m*, muscle cell; *n*, nerve tube; *nt*, notochord; *o*, otolith; *p*, pigment obscuring retinal cells.

The larval endostyle is relatively large and lies vertical to the main axis. The digestive tube is fairly well differentiated, while the peribranchial sacs, already fused mid-dorsally to form the atrial siphon, are united with the pharyngeal wall on each side and bear each three rows of perforate, definitive stigmata. There are eight or nine stigmata in each row, approximately the same as in fully grown zooids.

From the mid-ventral surface of the trunk of the tadpole, corresponding to the future posterior end of the individual zooid, a slender epidermal process grows out and forwards. From the anterior end of this there extends not only the three adhesive organs described above, but eight club-shaped ampullae, arranged in a ring around the more centrally placed adhesive organs.

After metamorphosis the ventral process expands and grows as the stalk of attachment into which extend the digestive canal, epicardium and heart. The anterior ampullae form an irregular organ of attachment, the adhesive organs having a transient function only.

Budding

During the winter, and to a very slight extent in summer months, growth of the colony occurs by budding. The method of bud formation (Figs. 4–6) is simple in the extreme, is found in other ascidians only in the diazonids, and is strikingly reminiscent of *Phoronis*.

The thorax slowly resorbs, a common phenomenon among ascidians. Associated with this, large cells or trophocytes, heavily laden with acquired food reserves, migrate posteriorly and congest the abdomen. They are similar to those that reach and congest the postabdomen of synoicids and the stolonic ampullae of the clavelinids, but here they are confined to the abdomen owing to the absence of any postabdominal extension and the lack of hypertrophy of the test vessels respectively.

Local growth activity of the epidermis then constricts the long abdomen into several oval masses, one constriction usually occurring below the stomach and two above. Only the epidermis plays an active part in the process, the inner tissues (epicardium, both limbs of the digestive canal, etc.) being passive and cut through by the invaginating outer layer.

The process of constriction is a local annular growth of epidermis inwards, the cells of the invading ring not only proliferating but exhibiting a change from cubical to columnar form. The inner end of each cell becomes greatly extended by a large vacuole. A similar vacuolated condition is typical of epicardial cells, at least in fragmenting abdomens. In both the vacuolar region is away from the exposed epithelial surface.

Regeneration occurs subsequently. In this process it is the epidermis that has the relatively minor role, producing only the epidermis of the new outgrowths. The internal structures, both anterior and posterior, are developed from the proliferating ends of that section of the epicardium contained in the isolated piece. The new branchial sac and gut loop thus formed are fitted on to

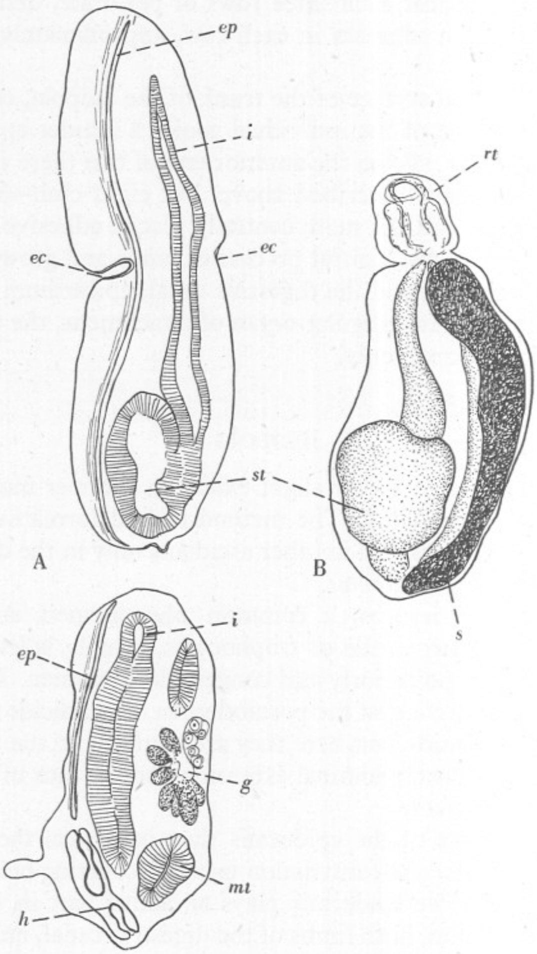

Fig. 4. *Archidistoma aggregatum*. Strobilation and regeneration. A, longitudinal section through strobilating abdomen, showing complete transverse division immediately below stomach, and incomplete constriction in middle part of anterior fragment (thorax resorbed); B, fragment containing original stomach etc., and part of distended sperm duct, regenerating new thorax from upper end of epicardium. *ec*, epidermal constriction; *ep*, epicardium; *g*, gonad; *h*, heart; *i*, intestine; *mt*, mid-intestine; *rt*, regenerating thorax; *s*, original sperm duct; *st*, stomach.

the surviving part of the original digestive canal. The food-laden cells produced during the resorption process become progressively smaller and more numerous, yielding their reserves in nourishing the growing tissues.

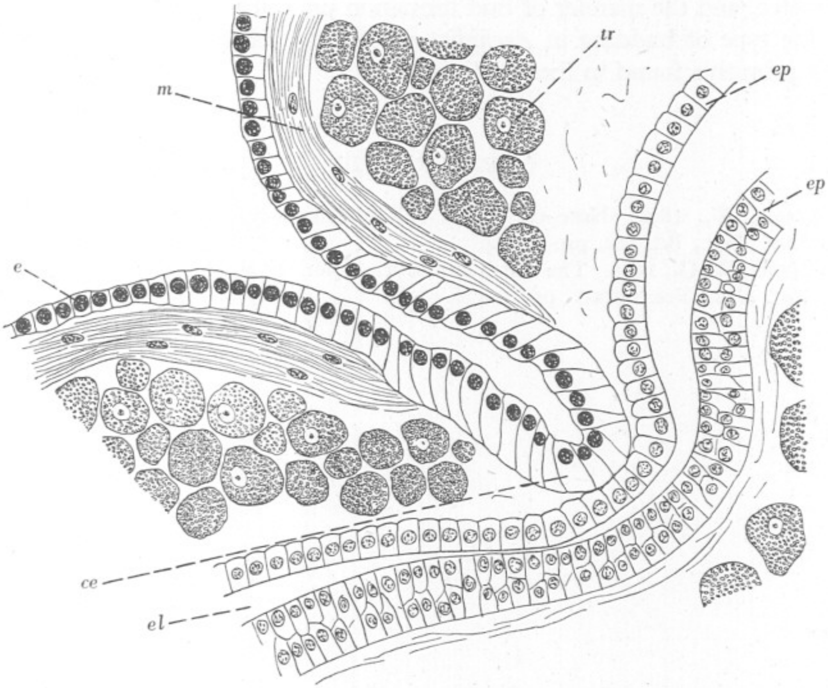

Fig. 5. *Archidistoma aggregatum*. Higher magnification of epidermal constriction shown in Fig. 4, showing epidermis cutting in through muscle and trophocyte layers and impinging on epicardium. *ce*, enlarged epidermal cells of constricting region; *e*, normal epidermis; *el*, lumen of epicardium; *ep*, lining cells of epicardium; *m*, retractor muscle; *tr*, trophocyte.

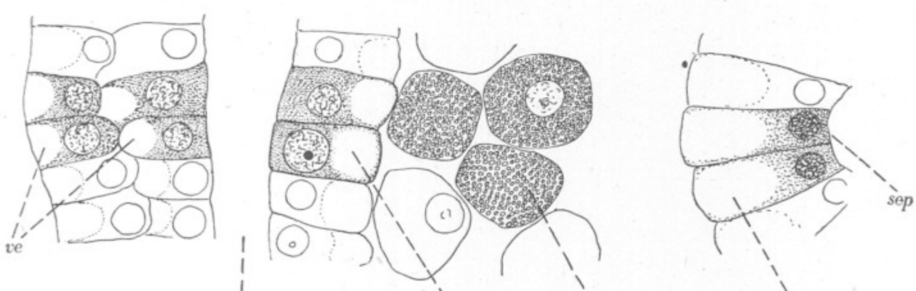

Fig. 6. Enlarged view of constricting epidermal cells and activated epicardial cells (× 1000), both showing vacuolization of the cells on the side away from the outer surface of the body and the epicardial cavity respectively. *el*, epicardial lumen; *sep*, outer surface of epidermis; *tr*, trophocyte; *ve*, vacuole of epicardial cell; *vep*, vacuole of epidermal cell in constricting region.

In so far as the general structure of the zooid appears to be comparatively primitive, and the manner of bud formation undoubtedly as simple as it can be, the type of budding in *Archidistoma* may reasonably be regarded as the most primitive found in the ascidians.

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