THE DEVELOPMENTAL HISTORY OF AMAROECIUM CONSTELLATUM. III. METAMORPHOSIS

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A description of metamorphosis in the compound ascidian, *Amaroecium constellatum,* is presented as the third in a series of papers covering its complete life history. The paper satisfies two needs. First, it helps to fill a gap in information about a tunicate widely distributed and well represented along the eastern shore. Second, it serves as a frame of reference for the presentation of the results of an experimental study on metamorphosis which forms the subject of another paper.

The extensive studies of Brien (1929, 1930), Brien and Blanjean (1939) on the embryology and metamorphosis of European ascidians and the excellent synthesis of Berrill (Tunicata, 1950), which reviews his own investigations and those of the earlier workers, provide a comprehensive background for the understanding of the process of metamorphosis in general. Such a background precludes the introduction of unnecessary details where the processes in Amaroecium parallel those already described in the other forms.

Absence in the literature of the complete life history of any one American tunicate is justification in itself for the completion of the present study.

Materials and Methods

Amaroecium constellatum was collected in the vicinity of Woods Hole, Massachusetts. The colonies were kept in shallow dishes in running sea water and put into a dark room at night to prevent the shedding of tadpoles shortly after sunrise. About twenty minutes after removing the dishes into the light tadpoles were shed and collected into Syracuse watch glasses for study. They were studied in the living state but opacity of body necessitated extensive use of stained and cleared specimens in whole mounts and serial sections.

The fixative that gave the best results was Schaudinn's solution heated to 60° C. and poured quickly and generously over the tilted dishes to insure the greatest possible extension of the animals. The Feulgen technique used on whole mounts produced very clear specimens at all stages, even those of the early periods that remain opaque in other stains. The sections were stained with alum haematoxylin and triosin. The photomicrographs were taken with a Zeiss photomicrographic camera.

Observations

Fixation of the larva

The tadpoles swim vigorously for a few minutes after being released through the cloacal aperture of the colonies. They alternately swim and rest in short periods, being attracted first to the light side of the dish where they rest, then swimming back to the side of the dish opposite the source of light (Grave, 1921). They swim for a time varying from a few to thirty minutes. At the end of the swimming period they leave the rim of water in the larger dishes and attach themselves to the side or bottom of the dish. In the Syracuse watch glasses they attach to the bottom, to the sides or to the surface film. Those that are attached to the surface film are lost when the watch glasses are put into running sea water.

At the moment of fixation the three adhesive papillae expel their secretion in an explosive action, and the attachment effected by this cementing substance may be broken without impairing a subsequent attachment at some other place. Upon explosion of the adhesive secretion the test vesicles detach themselves from the epidermal ridge, their point of origin, and move out into the tunic where they begin

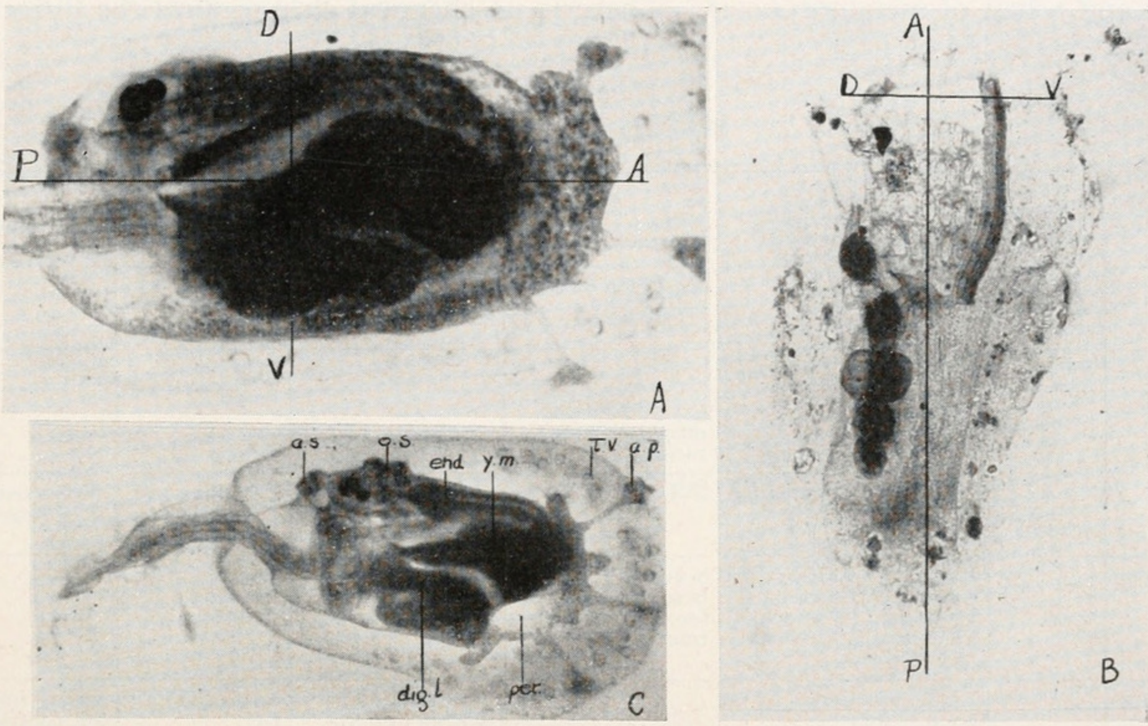

FIGURE 1. A. Tadpole at the moment of attachment showing larval axes. 200 ×. B. Ascidiozooid at the completion of metamorphosis, two days after fixation, showing adult axes. 50 ×. C. Tadpole, retraction of tail in progress. 125 ×. a.p., adhesive papillae; a.s., atrial siphon; end., endostyle; dig.l., digestive loop; o.s., oral or buccal siphon; per., pericardium; t.v., test vesicle; y.m., yolk mass.

to secrete additional tunicin. Within three minutes they are detached from the mantle and are distributed throughout the area of fixation. Withdrawal of the papillae from the surface and the additional tunic secreted between them produce a temporary scalloped appearance at the larval anterior end of the fixing ascidiozooid (Fig. 3, G). Since there is no addition to the test at its posterior half at this time the tunic flares broadly at its anterior half. The test vesicles rapidly lose their secretory capacity and wander out to the periphery where, eventually, they lose their identity. They function only during the period of fixation and they provide, with some questionable aid from the secretion of the adhesive papillae, a cementing substance in which the fixing larva can anchor itself during the establishment of its adult axis.

The beginning of the fixation process is marked also by retraction of the axial structures in the tail. When the tadpole comes to rest for attachment the papillae explode their contents, the tail stops twitching and, in a quick turn, arches itself into a final bend (Fig. 1, C). Within two minutes the contents of the caudal envelope buckle into one or two folds without disrupting the linear relationship of the notochord, neural tube and muscle cells. Simultaneously, the axial structures of the tail begin to retreat with smooth, flowing action into the posterior clear area of the tadpole's trunk where they are drawn into a compact mass (Fig. 3). When the caudal contents are pouring into the trunk, the cuticle becomes crinkled, giving support to Berrill's opinion (Berrill, 1947) that shrinkage of epidermis due to nutritional exhaustion may be the mechanical force initiating tail resorption. Each kind of cell retains its histological integrity and relationship during the first day and a half of metamorphosis. There is no chemical change until the differentiating processes of metamorphosis are accomplished. The epidermal cells at the tip of the tail together with the caudal mesenchyme form a dense cellular knob which plugs up the point of entrance of the resorbed mass (Fig. 2, A). It gradually moves away from the surface of the tunic as the caudal organs contract into their final disposition in the trunk. The empty envelope of the tail hangs on to the young ascidiozooid for two or three days and then falls off.

TABLE I

Time schedule of rotation

Time in minutes	Tail activity	Caudal structures	Caudal sheath	Adhesive papillae	Axis of fixation	Test vesicles	Rotation
0	Contractions stop			Explosion of adhesive jelly			
1	Flexure	Buckling				Migration	
2		Beginning of resorption		Rounded knobs retreated from surface			
3			Crinkling			Distribution through area of attachment	Body contractions begun
6		End of retraction. Epidermal knob at surface	Empty sheath attached to body	Stalk detached at base or at cup by force of body contractions		Located at surface of flared tunic	
8–10		Epidermal knob within trunk			Siphonal area twisted into upright position on base of attachment	Loss of identity	Strong rhythmic contractions in progress

Withdrawal of the tail and concurrent activities of related parts can best be summarized in a chart with time schedule included (Table I).

In many cases the tail is not resorbed. It continues to hang on the body during metamorphosis executing occasional quivers up to the second and third day of development (Fig. 4, B). Action of the test vesicles and the initiation of rotation movements are the same whether the caudal elements are retracted or retained in the attached tail. Nothing in the larval structures of the tail influences the metamorphosic process. The enzymatic action required for autolysis or phagocytosis of the tail tissues is in no sense responsible for the activities that initiate, favor, or effect the shift in axis from larval to adult state or for the differentiation of the adult organization.

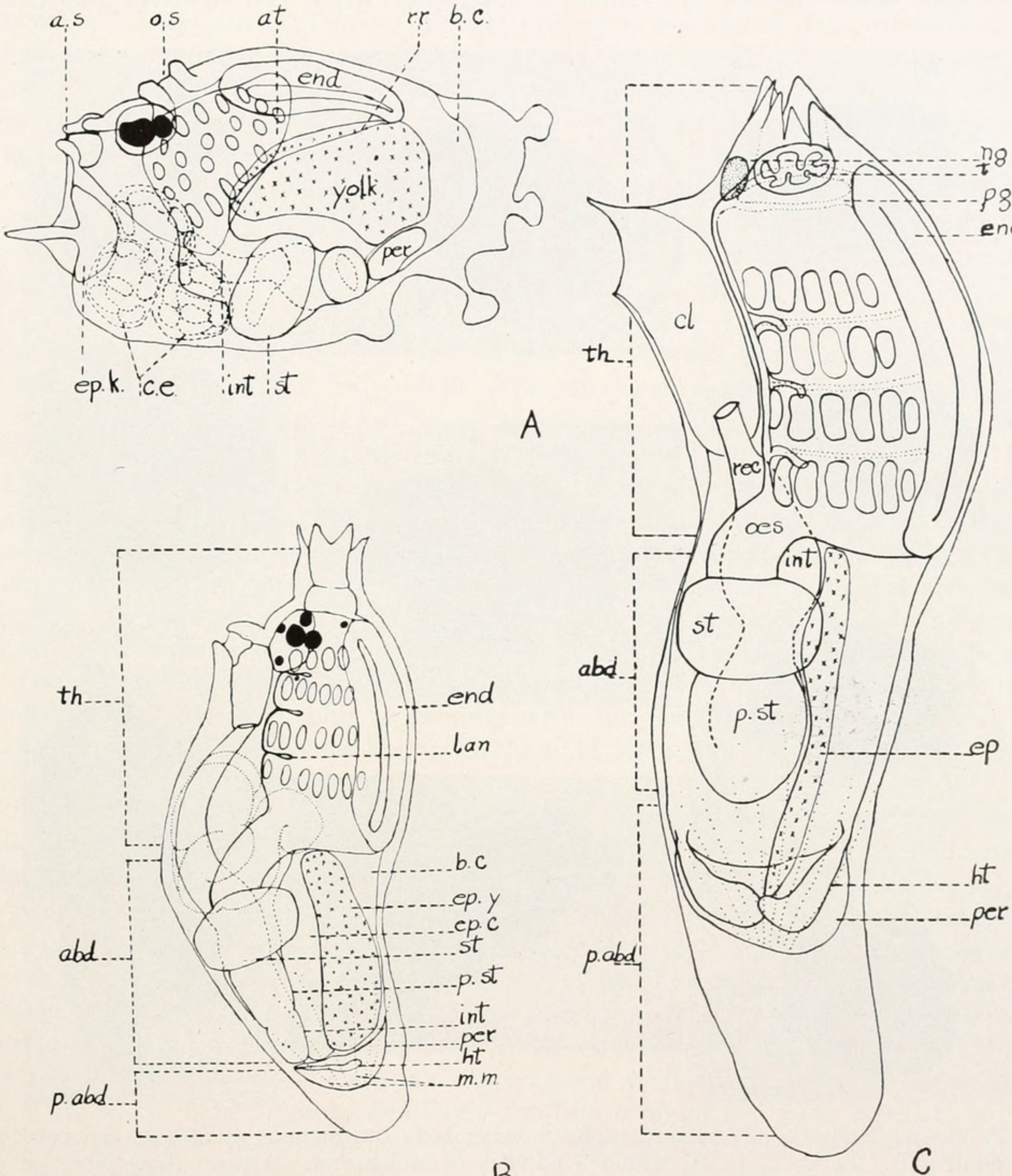

FIGURE 2. A. Tadpole at the beginning of metamorphosis. B. Ascidiozooid, 12 hours after fixation. C. Ascidiozooid during the third day of development. Abd., abdomen; a.s., atrial siphon; at., atrium; b.c., body cavity; c.e., caudal elements; cl., cloacal chamber; end., endostyle; ep., epicardial tube; ep.c., epicardial cavity; ep.y., epicardial yolk; ep.k., epithelial knob; ht., heart; int., intestine; lan., languet; m.m., muscles of mantle; n.g., neural ganglion; oes., oesophagus; o.s., oral siphon; p.abd., post-abdomen; per., pericardium; p.g., peripharyngeal groove; p.st., post-stomach; rec., rectum; r.r., retropharyngeal raphe; st., stomach; th., thorax. (*Camera lucida* drawings.)

Rotation

For clarification of terms used in ascidian metamorphosis it should be recalled that the antero-posterior axis of an Amaroecium larva is marked by the adhesive papillae at its anterior limit and base of the tail at its posterior limit; dorsal refers to the area of the siphons and ventral to the region where the digestive loop is lo-

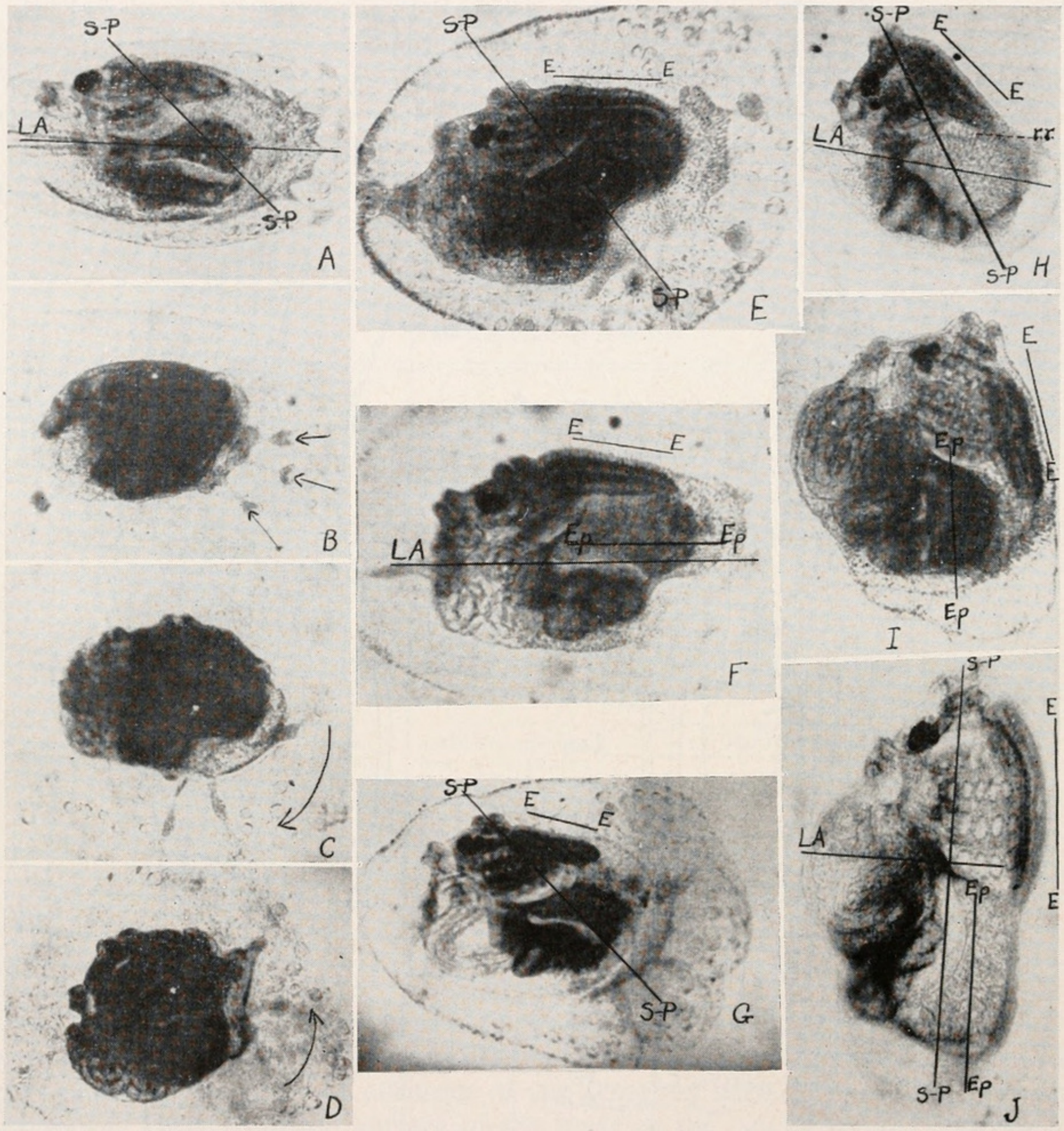

FIGURE 3. Series of photomicrographs showing body contractions of tadpole and rotation into adult axis. A, B, C, D, E, within 3 minutes of attachment. Arrows show direction of movement. F and G, 8 minutes; H, ½ hour; I, 2 hours; J, 3 hours after attachment. E–E, endostylar axis; Ep.–Ep., epicardial axis; La., larval axis; S–P, siphonal-pericardial axis; r.r., retropharyngeal raphe. About 100 ×.

cated (Figs. 1A, 2A). The antero-posterior axis of the adult Aplousobranchiata extends from the free siphonal area to the attached basal point (Figs. 1B; 2B, C). The pericardium is lodged in the basal tip of the post-abdomen. In the process of reorientation the yolk organ is projected into the lengthened post-abdomen and the pericardium becomes separated from the digestive loop without changing their original topographical relationship (Fig. 4D). It is clear, then, that the antero-posterior axis of the adult corresponds with the dorso-ventral axis of the larva whereas the larval antero-posterior axis is temporary. The dorsal region of the adult is associated with the position of the ganglion and, therefore, all organs on that side are referred to as being dorsal; ventral is associated with the endostyle and all organs on that side are referred to as being ventral. An examination of Figures 1C and 2A will afford adequate review of the structure of the free-swimming tadpole and relationships of the larval organs to provide a basis for the presentation of metamorphic activities.

The process of rotation begins from one to eight minutes after the explosion of the adhesive jelly and the detachment of the test vesicles. It is effected by a series of contractions by means of which the organs are rotated through an arc of about 110°.

A strong contraction moves around the anterior end of the larva from the tip of the endostyle to the base of the tail on the ventral side, involving all the structures in that region of the body. The force of the movement pulls the stalks of the papillae into a sharp curve, in most cases detaching the entire papilla from the surface and reducing it to a knob-like projection of the body wall (Fig. 3A, B, C, D); in other cases jerking the stalk away from the secretory cup which is left temporarily at the surface. At the height of contraction the knobs or stalks protrude from the ventral body wall (Fig. 3C). The digestive loop is telescoped into an irregularly spiraled mass pressed against the caudal elements; the endostyle and yolk organ are shifted from the position of forming a straight angle with the siphons, coinciding with the larval axis, toward the direction of forming a right angle with the siphons and coinciding with the adult axis (Fig. 3). The endostyle may be thrown into several deep folds in keeping with the general state of contraction in all the viscera. In the living animal nothing of its anatomy is discernible when the contraction is at its maximum.

The reverse action toward the dorsal side follows immediately upon the preceding movement but it is less forceful, the organs not recovering their former alignment. Movements, once begun, alternate rhythmically through a period varying from 6 to 12 minutes, the ventral contraction always being the more violent. After that period the contractions are less frequent and less marked, each one effecting a change in the direction of the adult axis. Since the siphons and caudal mass do not participate actively in the contractions and remain unchanged in actual position and in relationship to each other in the course of the movements, it is possible to consider the siphonal area as a fixed point with reference to the reorientation of the axis. The angle of total body rotation is about 70° from larval to adult axis; the endostyle moves through an arc of 90° to 100°; the pericardium and yolk organ through 90° (Fig. 3).

The adult axis is established within two hours or less after the first contractions begin. The activities that accompany the forceful movements of rotation are processes of growth and extension, the former being marked by numerous mitoses evident in the endostyle, digestive tract and cells of the body cavity. The siphons become more elevated and prominent, the oral siphon developing six blunt lobes (Fig. 4C). Both openings are filled with primary tunic and are non-functional. The tight coil of caudal elements bulges from the surface on the morphological dorsal


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Figure 4

side with the intestine pressed against it medially (Fig. 4A). The organism appears short and compact during the early stages of metamorphosis and becomes progressively more transparent as reorganization proceeds.

When rotation begins, the pharynx is limited in size, the peripharyngeal cavities pressing into it laterally and the nutritive endoderm occupying most of its space ventrally (Fig. 5A, B, C). With each movement of rotation the pharynx elongates in the direction of the developing axis and the peripharyngeal cavities, correspondingly, stretch into narrow chambers clasping right and left sides of the pharynx and meeting in the common cloacal cavity at the atrial siphon. The four rows of gill clefts come into evident view as the thorax elongates (Fig. 3G, I).[1]

Expansion of the pharynx is accomplished both by extension in length in the adult axis and by gradual expulsion of the yolk organ from its location in the floor of the cavity. The yolk organ is the mass of nutritive endoderm derived from the vegetal cells. It forms two thick-walled longitudinal furrows with an extension of the pharyngeal cavity below the yolk mass (Fig. 5G). The cavity is evident as a space between digestive loop and yolk in lateral views of the tadpole. In such views the whole mass appears wedge-shaped, its broad base facing the adhesive papillae, its narrow apex pointing toward the root of the tail (Figs. 1C, 3G).

A thin epithelium covers the surface of the yolk mass and is continuous with the walls of the pharynx. The epithelium of the upper surface from the tip of the endostyle to the oesophageal opening is called the retro-pharyngeal raphe (Brien, 1929) (Figs. 2A, 3G). With each contraction the raphe is shortened and the yolk organ is gradually eliminated from its larval association with the pharynx to a position posterior to it, the two assuming a linear relationship with no communication between them. Once the separation is effected, the structure can be called the epicardium. It continues to lie parallel with the digestive loop. The yolk organ in Amaroecium has, therefore, the same significance it has in the other Aplousobranchiata, so far described, where it is similarly transformed directly into the epicardial tube.

The heart and its surrounding pericardial sac, being located at the distal end of the U-shaped intestine and epicardium or yolk organ, are involved in the rotating process to the same extent as the adjoining organs. The motion of the heart is passive insofar as it remains unchanged in structure during this time but within the first hour it commences to beat rhythmically.

Three hours after the expulsion of jelly from the papillae all rearrangement of larval systems into adult organization has advanced to the state where the animal is easily recognized as a tunicate. Within five hours the body regions characteristic of tunicates are clearly marked off: the thorax with its prominent endostyle

[1] Compactness of body and pressure of atrium into pharynx are the explanation for two previous errors in reporting the number of gill clefts as 3 instead of 4 (Scott, 1946).

FIGURE 4. Continuation of the series showing assumption of adult body form and differentiation of adult organization. A, 5 hours; B, 12 hours; C, 18 hours; D, 24 hours; E, second day; F, third day after fixation. A, B, C, 150 ×; D, 130 ×; E, F, 60 ×. Animal has begun to take food in E, second day. Abd., abdomen; an., anus; d.l., dorsal languet; end., endostyle; ep.c., epicardial cavity; ep., epicardial tube; gl.st., glandular stomach; ht., heart; lan., languet; m.int., mid-intestine; oes., oesophagus; p.abd., post-abdomen; per., pericardium; p.g., peripharyngeal groove; pb.ph., prebranchial pharynx; p.st., post-stomach; th., thorax; y.m., yolk mass.

and four horizontal rows of clefts; the abdomen lodging the epicardium and U-shaped digestive tract; the post-abdomen, in rudiment, but containing the crescent-shaped pericardium curved around the distal end of the blunt epicardium. The larval sensory vesicle is still evident. The tail structures are unchanged and protrude from the wall of the abdomen (Figs. 3, 4).

Throughout the period of rotation and beginning about fifteen minutes after fixation, mesenchyme cells of the body cavity migrate through the body wall and pass into the tunic. They are stellate in shape and produce long fibrils which extend from one cell to another. They move slowly towards the periphery of the tunic which continues to form in advance of them. The primary test of the tadpole is a secretion of the mantle. Cells continue to migrate through the body wall and they spread through the tunic.

Part of the process of reorganization includes the adaptation for adequate feeding and, therefore, freedom of movement of the oral end of the ascidiozooid. This is accomplished by flexion of the pharyngeal region on the rest of the body into an upright position so that the siphons are erected into the water. It occurs early in metamorphosis, within ten minutes, and is an action independent of the rotation movements. At the end of metamorphosis the abdomen and post-abdomen lie horizontally on the substratum to which they are attached while the thorax projects freely into the water at a right angle to the posterior body.

Further differentiation of the adult systems

When the ascidiozooid has rotated into its final axis the organs are disposed in their adult organization. The subsequent process of metamorphic differentiation is concerned with the elaboration of the systems to their mature state.

The pharynx extends into prominent occupation of the thorax. Dorsal languets project into the cavity to the left side of the points of intersection of the three horizontal branchial sinuses with the dorsal longitudinal one (Figs. 2B, 5E, F). They are similar in structure and in location in all Aplousobranchiata. The endostyle is differentiated into glandular and ciliated regions, consisting of five sections on each side. The center of the furrow is occupied by the flagellated band. On each side are the two basal glandular masses of cells separated from each other by a deep but narrow inferior ciliated band. Above the crescent shaped masses is the very slender "superior ciliated band" connecting the third glandular area with the rest and permitting it to spread widely into the floor of the pharynx (Fig. 6H). Branchial clefts number seven in each half row, tapering in size from smaller ones at both ends to larger ones in the middle.

The buccal siphon projects prominently as a rounded eminence, its collar cleft into six blunt lobes, its aperture deeply indented to the velar base which is divided into tentacles. Below the velum is the pre-branchial chamber limited by the peripharyngeal grooves into which the ciliated funnel of the hypophyseal duct enters. The peripharyngeal grooves connect the endostyle with the dorsal lamina (Figs. 2C, 4D).

The atrial siphon is less conspicuously elevated from the surface and its shallow rim remains undivided. The aperture communicates with the common or cloacal chamber of the peripharyngeal cavities. Both apertures have a layer of tunic lining

FIGURE 5. A, Whole mount of tadpole at moment of fixation with lines to locate transverse sections in B and C. D, Whole mount of ascidiozooid 12 hours after fixation showing locations of transverse sections in G, H, I and J; E and F, longitudinal sections through D; K, Whole mount of tadpole at beginning of metamorphosis showing early constriction of digestive tract. A, 200 ×; D, 150 ×; K, 250 ×. at., atrium; br.s., bronchial sinus; c.e., caudal elements; end., endostyle; ep.c., epicardial cavity; ht., heart; int., intestine; lan., languet; m.int., mid-intestine; oes., oesophagus; per., pericardium; ph., pharynx; p.st., post-stomach; rec., rectum; st., stomach; y.m., yolk mass.

the collar but until the end of the first day of metamorphosis they are both closed with a plug of tunicin.

Between the two siphons lies the neural equipment, otolith and eye in a sensory vesicle to the right, neural ganglion and subneural gland to the immediate left and visceral or larval ganglion below them connecting with the neural tube of the tail mass (Fig. 3). The latter, still unchanged, presents a prominent bulge on that side of the body.

The epicardium and digestive loop lie posterior to the pharynx in the abdomen which is not, at this time, sharply demarcated from the thorax. Both structures end at the same level. Pericardium and pulsating heart form a clear crescent immediately behind them, the area of the body cavity enclosing them being the only indication of post-abdomen present toward the middle of the first day. The three body regions of tunicates are, therefore, evident but not completely developed (Fig. 4).

The remaining period of metamorphosis may be considered under the following activities:

(1) Body growth

In the course of two days the body completes its development into the adult form. The thorax expands into a transparent urn opening through a deep collared siphon crowned with six tapering lobes. The line of the buccal orifice curves through 90° to the atrial siphon communicating with the correspondingly expanded atrial cloaca. The abdomen assumes definite regional characteristics with elongation of the intestinal loop to its full length and reduction in width of the proximal epicardium. Early in the second day, 30 hours after fixation, presence of food and associated contents in typical pellet shape makes the abdomen opaque. Continued extension of the body in the long axis produces the attenuated post-abdomen. The major length of the epicardium with its light orange deutoplasm, the pericardium and closely packed cells of the body cavity impart a faintly mottled appearance to this region. A circular rim of tissue at the level of the pericardium and distal end of epicardium holds the ends of the muscle fibres of the mantle beyond which the post-abdominal tip is completely transparent (Figs. 2 and 4).

The processes of expansion and extension are attended by mitotic activity, especially in the more compact tissues like endostyle, digestive tract, and mesenchymatous cells. Epidermal cells show scattered divisions also but the fact that the tissue is spread into a thin layer makes the figures less evident.

(2) Differentiation of the digestive tract

The digestive tract constricts into regions. The oesophagus is a broad tube curving from a funnel-shaped pharyngeal orifice to the cardiac constriction. The stomach is a short, bulbous distension, abruptly narrowed posteriorly where it continues into the post-stomach or pyloric stomach (Figs. 2, 4, 5K). During the second day of development, growth in diameter of the stomach results in the formation of about 20 longitudinal crypts characteristic of the stomach of *Amaroecium constellatum* (Figs. 4F, 6D). Histological differentiation occurs and secretion is in progress when feeding begins.

The valve at the pyloric end of the stomach opens into the short, vertical limb of mid-intestine extending to the curve of the loop where a second constriction separates it from the final division of the intestine or rectum which ascends from that point to the floor of the atrium. When feeding commences, the mid-intestine compresses the mucus-bound contents into ovoid pellets and they retain that shape until their expulsion at the anus (Figs. 4F, 6A).

Histological differentiation is confined to the organization of epithelium typical

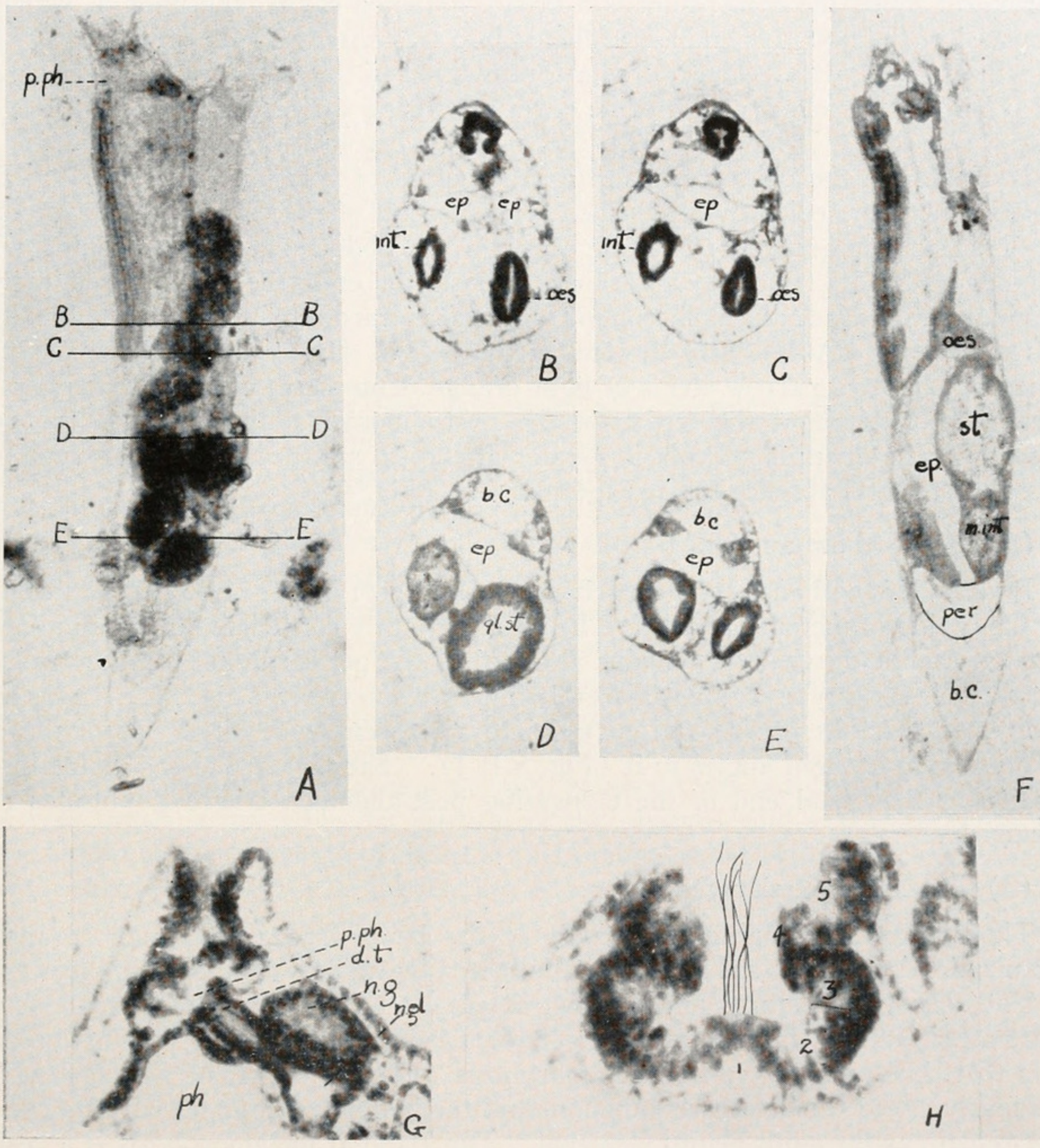

FIGURE 6. A, Whole mount of 2-day old ascidiozooid marked to locate transverse sections in B, C, D and E. F, longitudinal section through A. G, section through nervous system of adult. H, detail of endostyle with regions indicated. A, F, 100 ×; B, C, D, E, 150 ×; G, 450 ×; H, 1000 ×. B.c., body cavity; d.t., dorsal tubercle; ep., epicardium; gl.st., glandular stomach; int., intestine; m.int., mid-intestine; n.gl., neural gland; n.g., neural ganglion; oes., oesophagus; per., pericardium; ph., pharynx; p.ph., prebranchial pharynx; st., stomach.

of the various segments of the digestive tract; deep, slender columnar cells in oesophagus and mid-intestine; longitudinal glandular folds in the stomach; shallow columnar cells in the rectum. At a later stage, after budding has progressed, the pyloric gland of ascidians develops from the surface of the rectum to the pyloric stomach.

(3) The epicardium

When it is excluded from the pharyngeal cavity by shortening of the retropharyngeal raphe the epicardium is a broad, flattened cavity, the floor of which is composed of the nutritive endoderm flexed into two narrow clefts connected by thin epithelium (Fig. 5H). The roof is a continuation of the thin squamous epithelium that provides the yolk organ with its covering during the earlier stages of development. The cavity is identical with the one that becomes evident between the digestive loop and the deutoplasm during the period of rotation (Figs. 3, 4). Differentiation of the epicardium consists of elongation of the two yolk-lined furrows into long slender grooves extending through the abdomen and lengthening post-abdomen (Fig. 5E, F). At anterior and posterior ends the furrows retain their individuality but lack the yolk content and the epicardium is, therefore, double at both ends (Fig. 6A–F). By the end of the second day much of the yolk is consumed but the two narrow strips of light orange deutoplasm remain to identify the epicardium throughout the length of the post-abdomen a little beyond the pericardium. The quantity of yolk is greater at the tip and decreases in amount progressively to total absence toward the pharynx (Fig. 4).

(4) Pericardium and heart

Pericardium and enclosed heart change little except in size and position throughout the period of metamorphosis. The heart is tightly constricted in its middle and opens at each end, where it remains attached to the pericardium by a narrow neck into the sub-endostylar and perivisceral sinuses respectively (Fig. 2C). The pericardium is spacious in size and curved into a crescent with distal tips of the epicardium lying at right angles to it (Fig. 5 I, J). The pericardial sac maintains its location at the distal end of the elongating post-abdomen where it pulsates conspicuously.

(5) Degenerative changes

Those organs belonging exclusively to the larval action system or "transient organization" (Berrill) are gradually eliminated by various disintegrating processes.

The sensory structures and associated ganglia begin to disintegrate about five hours after fixation, fragments of the pigment being found in any part of the body from that time until the end of metamorphosis (Figs. 4, 5). The neural ganglion, previously called the definitive ganglion, and the hypophyseal gland with its ciliated duct remain as the adult neural elements. The gland at metamorphosis is a vesicular enlargement under the ganglion, and at its posterior end. It opens into the basal chamber of the buccal siphon by a ciliated duct with a funnel-shaped aperture called the dorsal tubercle. The region of the oral siphon is referred to as the prebranchial pharynx by Berrill. It lies between the peripharyngeal bands and the velar tentacles (Fig. 6G).

The caudal mass begins to break up during the latter part of the second day. The chordal, muscle, and neural cells lose their identity and clumps of them are scattered about through the body cavity. After their disruption the body is uniformly slender except for the expanded thorax holding itself erect on the recumbent abdomen and post-abdomen (Fig. 4).

Discussion

Descriptions of metamorphosis in ascidians are confined, in general, to considerations of the morphological aspects of the reorganization of larval systems into the adult. Explanations have been proposed to account for rotation on the transverse axis in various degrees of angles from 90° to 180°. Seeliger (1885) describes in Clavelina a pocket of epidermis depressed between the papillae and the oral siphon which is everted to the surface to assist in carrying the siphons through the arc to their position opposite the point of attachment. The pocket is "spare" tissue provided during embryonic development for the lengthening of the body on that side.

Observation of metamorphosis in Amaroecium in the living state is a convincing demonstration of the active role played by contractions of the body in reorienting the animal on its axis. The muscle strands developed in the mantle of the larva effect the movements.

The larva ingests neither food nor water and, therefore, the contractions can alter the shape of the flexible organs without impairing respiratory or digestive activities. All parts of the digestive tract are involved to the extent that the enteron is literally pushed into a tortuous mass toward the oesophageal funnel. The oesophagus and oral siphon retain their relationship; the cloacal chamber of the atrium and the anus retain their relationship; the convoluted loop of stomach and intestine, at the end of the contraction period, again extends itself in length but into a straight angle with the siphon, having rotated thus through an angle of 90°.

The endostyle is a densely cellulated structure in the tadpole and plays no active role in the contractions. It may buckle into one or two sharp folds and it always becomes arched during the height of the torsion but it retains its rod-like appearance. It is moved in hinge-fashion through an arc of 90° with the siphonal region. The yolk organ is inflexible but dilation of the pharynx and extension of its wall to the length of the endostyle in the new axis presses it out of the pharyngeal cavity into the space cleared by the telescoping effect of contraction on the enteric loop. Brien (1930) refers to the exclusion of the deutoplasmic mass from the pharynx as a shortening of the retro-pharyngeal raphe in Fragarium. He does not refer to the part played by the contractions of the mantle as initiating the process but the change in relative length of the epithelium between oesophagus and tip of endostyle is the same in both ascidiozooids.

The differentiation or dedifferentiation of the epicardium from the yolk mass is similar, also, in both animals, Fragarium and Amaroecium, being a direct conversion of the nutritive yolk into the slender, yolk-retaining epicardial tube of the abdomen and post-abdomen. It may be a characteristic phase of metamorphosis in the heavy-yolked Aplousobranchiata. In general, the epicardium of ascidians is produced by evaginations of the pharyngeal floor.

During the progress of metamorphosis it is possible to see the migration of mesenchyme cells through the mantle into the tunic. They move slowly through the tunicin until they establish a loose network with their long processes. The cells constantly change shape and the tunic becomes thicker but it is impossible to ascertain whether the cells are adding part of the secretion from their activity. Berrill (1950) is of the opinion that all tunic is secreted by the mantle or epidermis. An analysis of some experimental work which will follow in a subsequent paper leaves no doubt that the mantle is the seat of secretory activity early in the period of metamorphosis. This is true particularly in the area of attachment where the secretion effects adhesion to the substrate. The adhesive papillae provide for the preliminary attachment which is of transitory character, the test vesicles provide some of the cementing tunicin which is effective during the early stages of metamorphosis. Their secretory function is of short duration and is restricted to the region distal to the siphons. It seems that the test vesicles provide attachment for the contraction period when anchorage is required for the strong body movements of rotation and erection and before the mantle commences its secreting activity. The fact that the mesenchyme cells are in full march through the mantle when the tunic thickens uniformly over the surface of the ascidiozooid indicates that the final tunic may be the result of the combined activity of mantle and mesenchyme cells of the body cavity.

The points of discussion are of no controversial value but they clarify certain phases of metamorphosis in tunicates that have not been considered previously and, also, they leave another point, the origin of the tunic, to further study and analysis.

Summary

1. Metamorphosis in Amaroecium commences with the explosive release of secretion by the adhesive papillae and the steady, but rapid withdrawal of the caudal contents into a compact identifiable mass at the posterior end of the tadpole's trunk.

2. Reorientation of axes is initiated by strong contractions of the larval body which mark the beginning of reorganization in the internal organs.

3. The digestive loop, endostyle, yolk mass, and heart rotate through an arc of about 90° to their adult alignment by the action of the contractile force.

4. The pharynx expands and increases in depth being accompanied in its extension by the atrial or peripharyngeal cavities, the four rows of gill slits coming prominently into view.

5. Epicardial tube is differentiated directly from the yolk mass.

6. Typical thoracic, abdominal and post-abdominal regions of the tunicate body are developed by the end of the first twelve hours.

7. Ganglion and subneural glands are transformed into the adult nervous system while the products of disintegrating sensory pigment are scattered through the body.

8. Cytolysis of the caudal tissues begins after the adult organization has completely replaced the larval organization.

9. Reorientation of axes is accomplished within an hour after fixation of the larva; metamorphosis may be considered as completed within forty-eight hours.

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