Studies in Tunicate Development. Part V. The Evolution and Classification of Ascidians

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II—Studies in Tunicate Development

Part V—The Evolution and Classification of Ascidians

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Contents

I—Introduction

The purpose of the present paper is twofold. The development and variability of the heart, pericardium, and epicardium throughout the group will be described, while an attempt is made to trace the probable course of evolution within the ascidians and to base upon it a revised classification as it concerns the major divisions of the class.

The mode of origin of the heart and pericardium has long been recognized as one of the strong links between the tunicates and vertebrates, while the epicardium is a highly significant structure assumed by many to be concerned primarily with budding. Current classifications of ascidians, such as those of Lahille and Seeliger, are based upon the nature of the branchial sac, an insecure foundation since branchial structure is to a great extent an expression of the size of the organism; so that a more general basis is desirable.

A preliminary description is given of the adult structure and the development of *Ciona*,* inasmuch as there is good reason to believe that this genus represents

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* References to original articles are not given for all species in order to save space, as most are well known.

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closely the ancestral condition and there is no incontrovertible evidence of specialization.

II—Material

The material was studied in the form mostly of whole mounts and serial transverse sections. Frequently the circulatory system and other aspects of adult morphology were studied in the living animal.

Of the various genera described, *Ciona*, *Diazona*, *Ascidiella*, *Clavelina*, *Pycnoclavella*, *Archidistoma*, *Distaplia*, *Morchellium*, *Diplosoma*, *Botryllus*, *Polycarpa*, and *Molgula* were obtained while at the laboratory of the Marine Biological Association at Plymouth; *Perophora*, *Ecteinascidia*, and *Eudistoma* at the Bermuda Biological Station, to which institutions acknowledgments are made. *Euherdmania* was supplied by the Pacific Grove Biological Laboratory, *Rhopalea* by the Zoological Station at Naples; while *Tylobranchion* and *Colella* were examined through the courtesy of the British Museum (Natural History).

III—Morphology and Development of *Ciona*

Ciona is a comparatively large solitary ascidian. It is oviparous and without power to bud. Transverse blood vessels pass between the numerous rows of stigmata from the subendostylar vessel to the dorsal vessel. On the inner side of the branchial or pharyngeal wall longitudinal blood vessels occur, and where they cross the transverse vessels there is formed a bifid papilla projecting into the branchial cavity. Both Lahille (1890) and Seeliger (1893–1907) have grouped *Ciona* with all such species bearing branchial papillae to form respectively the order Phlebobranchiata or Dictyobranchiata, comprising in reality a decidedly heterogeneous assortment.

The gut is coiled posteriorly to the branchial sac as shown in fig. 1, while the gonads lie in the loop of the gut and their ducts accompany the intestine to the atrial siphon. The retractile muscles of the siphons are formed by six muscle bands on each side, inserted posteriorly near the point of attachment of the individual to the substratum. The attachment itself is effected by numerous extensions of the test, this last material having been shown by Brien (1930) to be an external collagen secreted by mesenchyme cells that migrate through the epidermis.

The heart is a longitudinal invagination of the tubular pericardium, and opens at one end at the base of the endostyle into the sub-endostylar vessel, and at the other into vessels passing over the wall of the stomach. It is longer than the distance between these two regions and forms a V-shaped tube. Vessels in the test contain two channels separated by a mesenchymatous septum (*cf.* Ärnbäck and Brien, 1932), blood flowing from one end of the heart to the terminal swelling of the vessel and returning to the other end.

The epicardium is in the form of a right and left perivisceral chamber opening by small apertures into the posterior end of the pharynx, fig. 1B. It is possible that they have a general excretory function, for they allow water to bathe directly the pericardium, gonads, stomach, and intestine, no other excretory structures being known in *Ciona*.

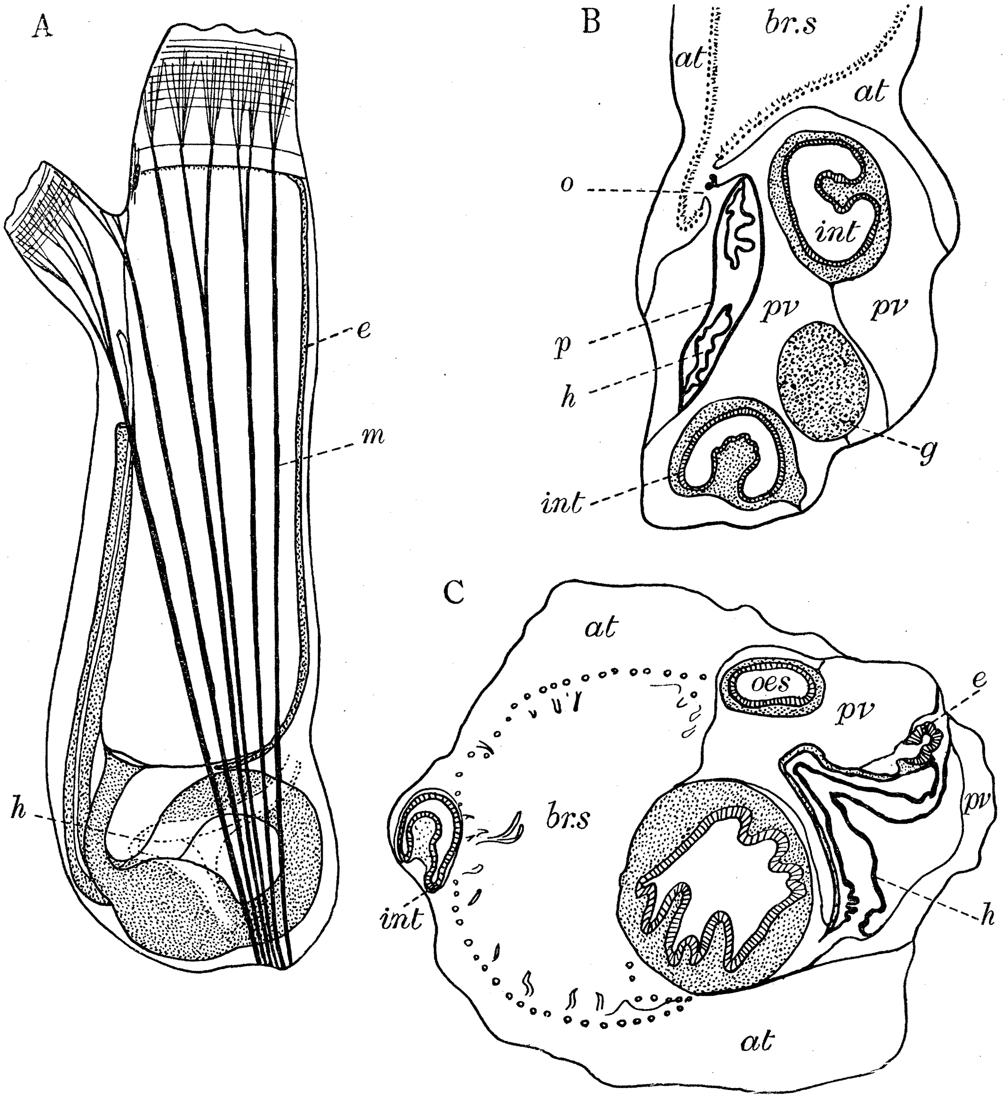

FIG. 1—Morphology of *Ciona intestinalis*. A, adult from right side, showing muscle bands, digestive canal and heart; B, vertical section through base of adult showing heart and pericardium, digestive tube, gonad, and the openings of the perivisceral sacs (epicardia) into the pharynx; C, transverse section through small (5 mm) individual, showing various parts of digestive canal, and the heart with its openings to the stomach and endostyle. *at*, atrium; *br. s*, branchial sac; *e*, endostyle; *g*, gonad; *h*, heart; *int*, intestine; *m*, muscle; *o*, opening of epicardium to pharynx; *oes*, oesophagus; *p*, pericardium; *pv*, perivisceral sac.

In the course of development a tadpole larva is formed, and after its metamorphosis a small peculiar post-larval ascidian. The development and nature of the tadpole has little significance in the present connexion, for in its essentials there is a remarkable uniformity throughout the ascidians, and tadpole variability, while of confirmatory value, is of secondary importance. The post-larval ascidian, however, differs in certain important respects from the adult. Correlated with its minute size there are but two protostigmata on each side in place of many rows of definitive stigmata. Also, in place of the single atrial siphon of the adult are two peribranchial siphons. These fuse eventually to form the atrial siphon at a time when the protostigmata have increased in number to six on each side and are becoming divided into a corresponding number of rows of stigmata. The pericardium develops as an evagination from the ventral wall of the pharynx, and the heart is formed by the infolding of the inner wall of the pericardium. At a slightly later stage when the protostigmata are functioning, the epicardia develop as two posterior evaginations from the pharynx to form the enveloping perivisceral cavities, with relatively wide openings into the pharynx, fig. 3B. At this stage the heart and pericardium is a short, straight tube passing from the base of the endostyle to the posterior end of the stomach, and only as the individual grows in size does the heart elongate. Since its two ends are fixed in position, elongation necessarily induces the V-shape characteristic of the adult, fig. 2.

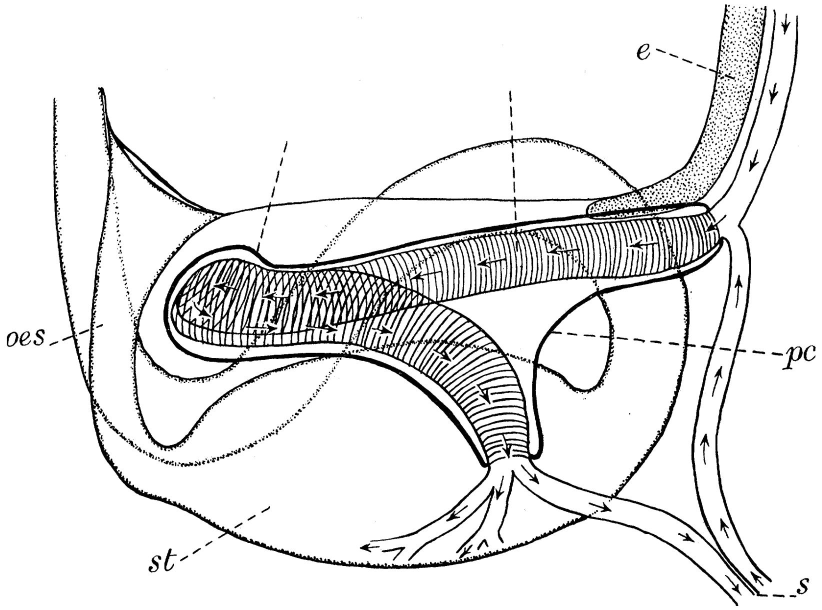

FIG. 2—An enlarged view of the heart and pericardium of *Ciona* showing the relationship with the intestine and endostyle. *e*, endostyle; *pc*, pericardium; *oes*, oesophagus; *s*, septum; *st*, stomach.

In these early stages a structure is present that is absent in the adult. This is the post-abdomen, an epidermal stalk into which extend the retractile muscles of the siphons.

When the individual first functions as an ascidian the post-abdomen contains only mesenchyme in addition to the muscle fibres, but as growth proceeds a vessel descends from each end of the heart, the two separated from one another by a mesenchymatous septum.

With further growth the post-abdomen shrinks and is represented in the adult only by the vessels of the test. In two rare varieties, however, of *Ciona intestinalis*, namely *v. longissima* and *v. gelatinosa*, according to ÄRNBÄCK and BRIEN (1932) the post-abdomen survives in the adult and may contain an extension of the left epicardium (perivisceral cavity) in addition to the muscle strands and blood vessels.

The above development is considered to be primitive. The adult structure to which it leads is also considered to be primitive, although there is the possibility that the stalk (post-abdomen) was once inhabited by the viscera as in many other forms. The V-shape of the heart and pericardium suggests this, but, as already described, that shape can readily be explained by the exigencies of growth. The power of budding may have been lost or may never have been acquired. If it once was present there is no reason to believe that it was of the type characteristic either of the perophorids or of the polystyelids, and the only likely method of budding in *Ciona* would be the abdominal constriction typical of the diazonids, distomids, and synoicids (*cf.* BERRILL, 1935, *b*). This could have occurred only if the gut-loop had descended into the stalk, and if the loop has always existed in the same relative position as it occurs in living *Ciona*, then abdominal budding is inconceivable. These two conclusions are therefore connected. If the position of the cionid gut is primitive, the absence of budding is also a primitive feature. If the position in the adult is secondary, then it is quite possible that abdominal budding once occurred. The position of the gut, however, is the same in the newly functional individual as it is in the adult, and the fact that this is the position typical of very young diazonids *before* the gut-loop descends into the stalk in those forms seems to confirm its primitive nature.

Thus no feature of the development or adult anatomy of *Ciona* can with certainty be said to be specialized, while there is some evidence that every such feature is a primitive character.

From this standpoint the remainder of the ascidians will be discussed.

From a cionid-like ancestor ascidians have apparently evolved in two main directions, one involving the descent of the viscera into the stalk, the other the shifting of the viscera forwards along the branchial wall. The first of these evolutionary trends gives rise to the order Aplousobranchiata (LAHILLE) or Krikobranchiata (SEELIGER) together with the family Diazonidae. In fact, HARTMEYER (1923) saw such a close relationship between the diazonids and *Ciona* that he included them within one family.

IV—DESCENT OF THE VISCERA

Diazona, like *Ciona*, is an oviparous genus. It is the only genus with the faculty for budding that is so. It produces tadpoles like those of *Ciona*, while the post-larval ascidian can be distinguished from that of *Ciona* only by the relative larger fixation stalk, a structure that grows from the anterior end of the tadpole. It is shown in fig. 3E, and there can be seen the two peribranchial siphons, the extensions of the muscle fibres to the base of the stalk, and the short tubular heart extending from the posterior end of the stomach to the base of the endostyle. The gut is coiled beneath the branchial sac as in the young and adult of *Ciona*. At this stage the perivisceral sacs, again as in *Ciona*, are forming from the posterior end of the pharynx and have wide openings forward.

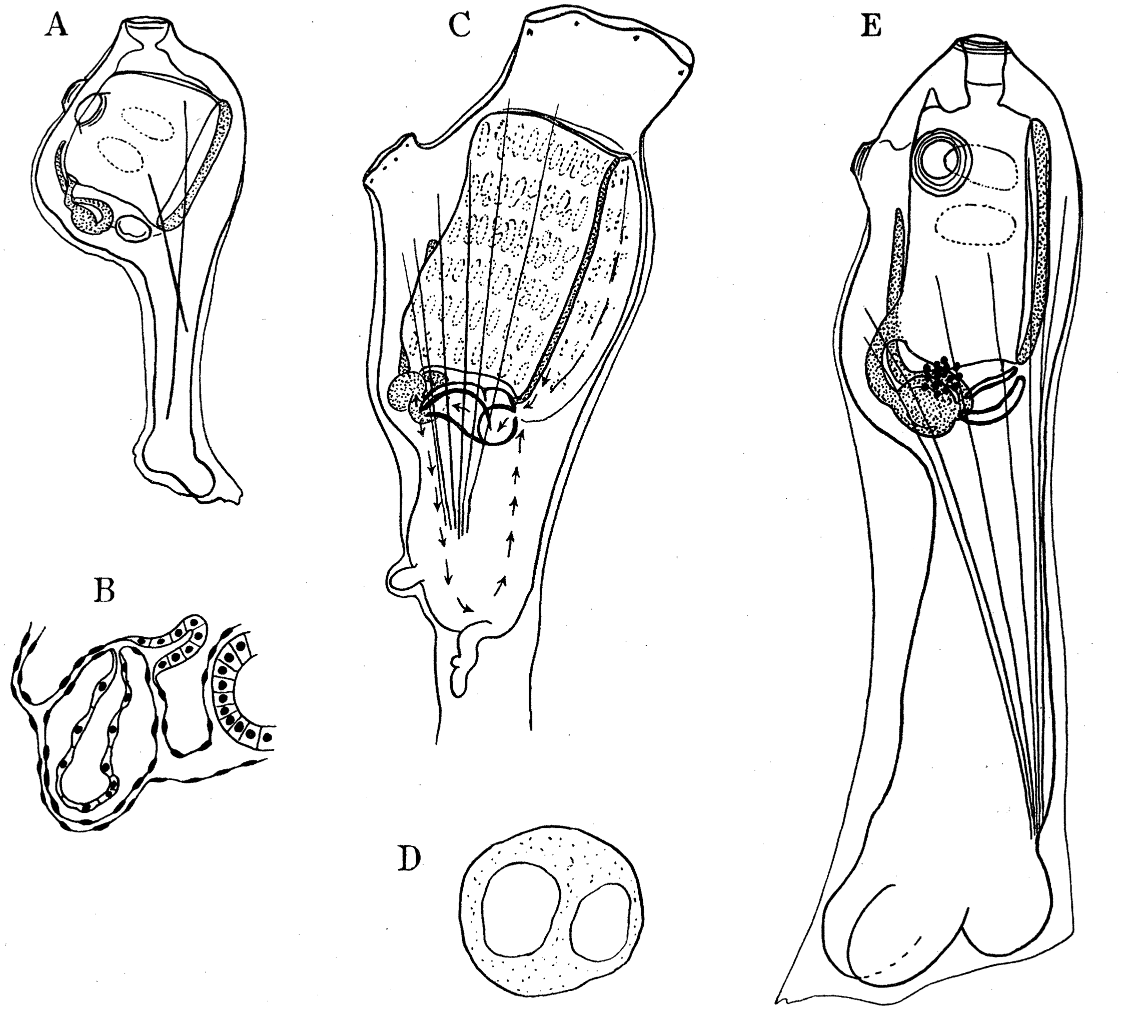

Fig. 3—The post-larval development of *Ciona* and *Diazona*. A, newly-functional *Ciona*, showing stalk with muscle fibres, the pair of peribranchial or atrial siphons, two protostigmata, and heart and gut-loop ; B, vertical section of similar stage (after Kuhn), showing the pair of perivisceral sacs or epicardia descending around the pericardium and heart ; C, older *Ciona* with protostigmata divided into rows of definitive stigmata, single fused atrial siphon, circulation in stalk, and bending of heart ; *D*, cross-section of stalk of stage C, showing blood sinuses and intervening mesenchymatous septum ; E, newly-functional *Diazona*, similar to *Ciona* except for the hypertrophied stalk, showing non-descended gut-loop and simple straight heart. The illustrations are drawn to different scales, the stigmata in reality being approximately all of the same size.

Nothing is known of the development between this stage and the adult condition, although there is no doubt that the peribranchial siphons fuse to form the median atrial siphon, and the protostigmata give rise to the rows of definitive stigmata, as in *Ciona*. Internal longitudinal vessels are present in the adult, with papillae at their junctions with the transverse vessels. Apart from size the thorax of *Diazona*

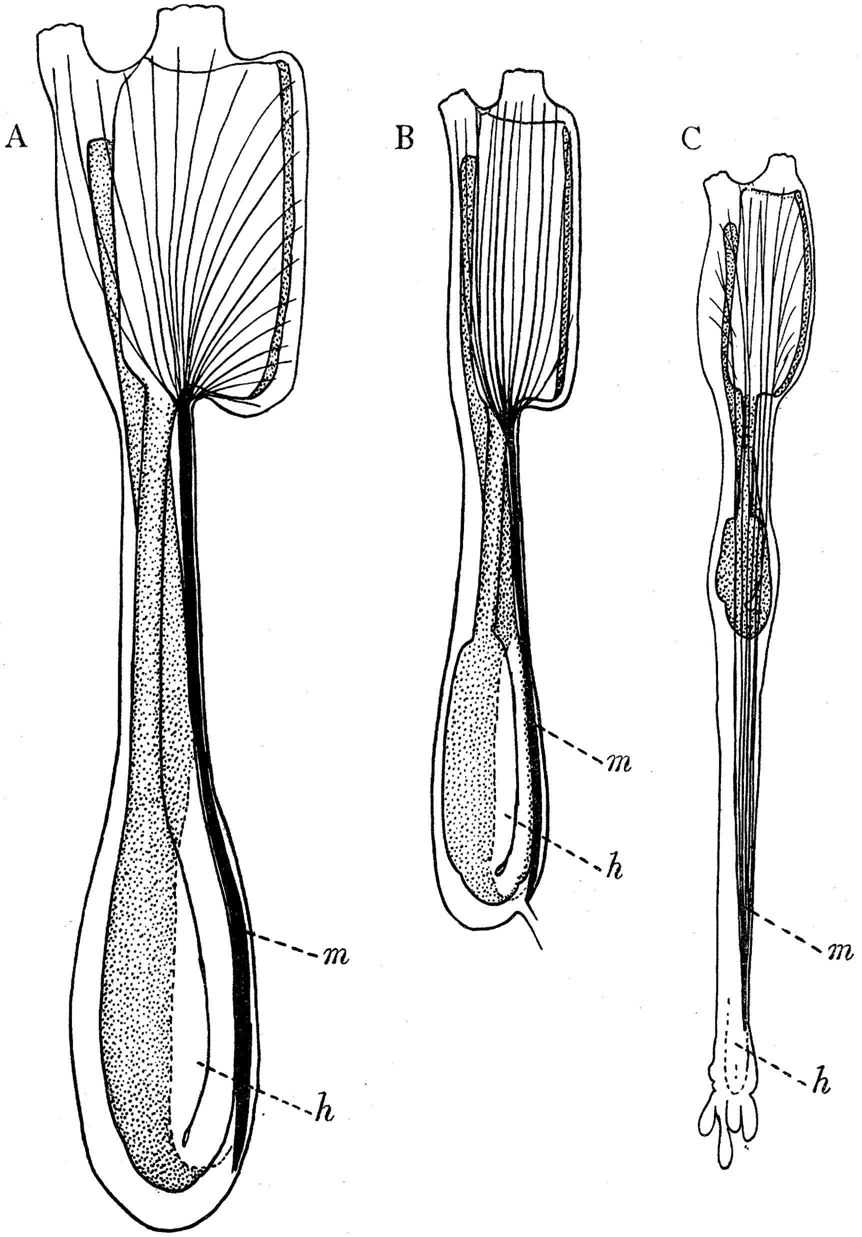

FIG. 4—A comparison of adult zooids of *Rhopalea*, *Diazona*, and *Tylobranchion*, to show the musculature, the descent of the viscera into the stalk, and the relative position of the heart. In *Tylobranchion* the stalk is elongated, the posterior part forming the so-called " post-abdomen " containing heart, muscle, epicardia, and gonads. (Drawn approximately to the same scale) : *h*, heart ; *m*, muscle band.

and *Ciona* are remarkably alike, and this is the basis of their union within one family by Hartmeyer. In the abdominal region, however, the course of development must be markedly different, for in the adult the gut is no longer coiled beneath the branchial sac as in all stages of *Ciona* and in the post-larval stage of *Diazona*, but has descended to the base of the stalk, carrying with it the heart, gonads and perivisceral sacs. The test vessel persists as in *Ciona*, and is composed of a blood sinus from each end of the heart separated by a mesenchymatous septum that seems to take origin from the pericardium. These relationships are to be seen in fig. 5E. The two ends of the heart are still related to the stomach and the base of the endostyle, but the heart itself is more definitely V-shaped with the acute angle

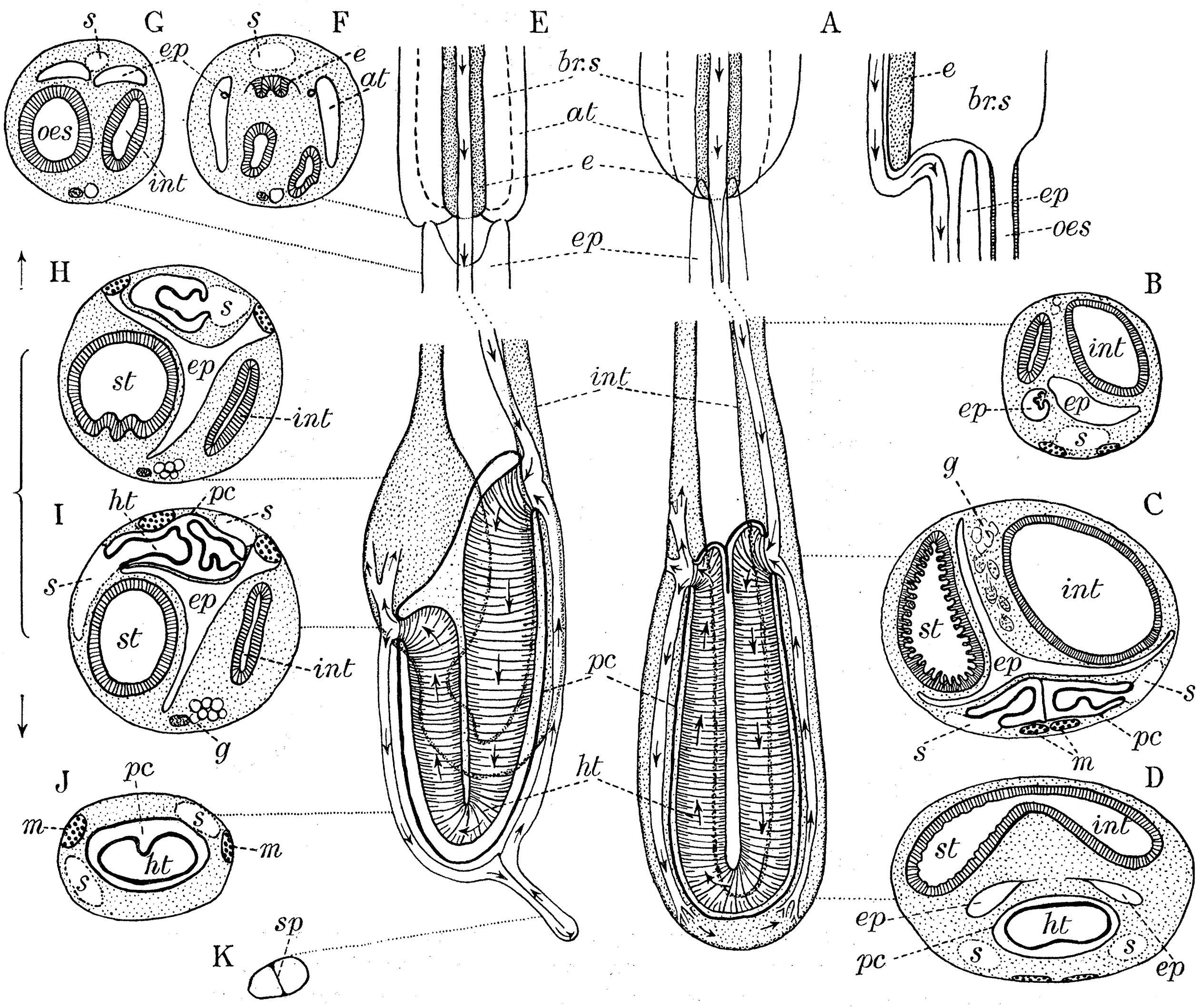

Fig. 5—Transverse sections and reconstructions of the post-branchial region of *Rhopalea* and of *Diazona*. A, reconstruction; B, C, D, sections of *Rhopalea*; E, reconstruction; F–K, sections of *Diazona*. *at*, atrial cavity; *br. s*, branchial sac; *e*, endostyle; *ep*, epicardium; *g*, gonad; *ht*, heart; *int*, intestine; *m*, muscle band; *oes*, oesophagus; *pc*, pericardium; *s*, blood sinus; *sp*, mesenchymatous septum; *st*, stomach.

forming at the base of the stalk. A further change is to be found in the nature of the perivisceral sacs. These descend with the rest of the viscera and lie between the gut-loop and the heart, but instead of remaining separate from one another and with openings into the branchial sac or pharynx, the two sacs fuse to form a single chamber through the greater part of their length. The original openings close, although one of the anterior horns gains a secondary opening not into the pharynx, but into the peribranchial cavity, fig. 5F. This again suggests an excretory function of the perivisceral cavity (or epicardium), especially as secretions into the lumen are noticeable in *Diazona* and many other forms.

Diazona is a colonial form, and buds by a process of abdominal constriction, the constrictions being an epidermal phenomenon, while the cells responsible for the reorganization and regeneration come from the wall of the epicardium (Berrill, 1935, *b*).

Rhopalea in some ways is a link between *Diazona* and *Ciona*. It is usually solitary and intermediate in size between the two forms, the power to bud being suspected but not proved to exist. Its general structure is shown in figs. 4 and 5. It differs from *Diazona* only in that the two limbs of the heart are of approximately equal length, there is no ventral test vessel, and there is no secondary opening of the epicardium into the peribranchial cavity.

V—Influence of Dwarfing

The cionid-diazonid stock undoubtedly represents the primitive ascidian type, and, as already suggested, there is evidence that the cionid condition is more primitive than the diazonid, rather than derived from it.

The remaining diazonid genus is *Tylobranchion*, a rare form of the South Atlantic. The zooids and colonies are smaller than those of *Diazona*. This question of absolute size has already been discussed (Berrill, 1935, *a*, *b*), but it may be noted here that reduction in size of a colony reduces the volume more than the surface, so that reduction tends to cause congestion of the posterior ends of the zooids embedded in the mass of test. In order to overcome such posterior crowding, two modifications appear. One is the extreme shortening of the zooid, the other is its relative elongation so that it becomes proportionately slender. Thus a zooid of Tylobranchion is much more cone-shaped than is one of *Diazona*, and this has been brought about by a growth of the stalk between its base and the posterior end of the abdomen. Into this post-abdominal stalk extend the muscle fibres, the gonads, the epicardium, and the heart, so that the zooid as a whole becomes relatively longer and narrower. Rather than a growth of the stalk, into which certain organs extend, it should be imagined that the whole stalk and contents extend posteriorly with the exception of the intestinal loop.

In all species of *Tylobranchion* the transverse vessels bear bifid papillae, but in none do they unite to form longitudinal vessels (Ärnbäck, 1926). This may be correlated with the small size of the zooids, for in *Rhopalea norvegica* (ÄRNBÄCK, 1925) the papillae are united to form longitudinal vessels in an individual of 20 mm length, but in one of 14 mm there are merely transverse vessels bearing bifid papillae. In other words, only when individuals exceed a certain size do the papillae unite to form longitudinal vessels. In *Rhopalea* this critical size lies between 14 and 20 mm length, and since the average size of the three known species of *Tylobranchion* is about 5, 11, and 14 mm respectively, the absence of longitudinal vessels is understandable. This subject has been emphasized since the presence of longitudinal vessels and the secondary papillae born by them has been used to define the order Ptychobranchiata (Phlebobranchiata). If dwarfing involves their loss or non-development, then obviously they have limited diagnostic value.

Among the Krikobranchia (Aplousobranchia) there are many types; were it not for the absence of inner longitudinal vessels in the branchial wall they would be recognized as being closely related to the diazonids. Thus the distomids, *Archidistoma* and *Eudistoma*, can be regarded as dwarfed diazonids. The stalk contains the gut-loop, gonads, heart and epicardium, as in *Diazona*. The epicardia are fused and have lost the openings into the pharynx. There is a narrow vessel to the test, containing a mesenchymatous septum; and budding is by abdominal constriction (BERRILL, 1935, *b*). The zooids and colonies are very small and are, in fact, dwarfed diazonid types, there being associated with dwarfness a loss of the internal longitudinal vessels and the papillae that give rise to them, and as in most dwarfed ascidians, the eggs are few and large, giving rise to comparatively elaborate tadpole larvae.

Reconstructions of the abdominal region of *Archidistoma*, *Eudistoma*, and *Pycnoclavella* are to be seen in fig. 6, showing the relative positions of gut-loop and epicardium, and the U-shaped heart, which in these very small zooids is comparatively short.

Closely related to the above distomids are *Distaplia* and *Colella*, two forms that have further elaborated the colonial state. The zooids are again very small and short, but become arranged in the colony in definite systems. They are also specialized in their method of budding (*cf.* BERRILL, 1935, *b*). Structurally they differ from *Archidistoma* or *Eudistoma* in that the ventral test vessel is enormously hypertrophied and contains wide blood channels, separated as in previously mentioned genera by a mesenchymatous septum arising from or near the pericardial wall. The vessel has a twofold significance. In *Distaplia* it is sterile, buds arising in the oesophageal region, but in *Colella* the stolon constricts into buds and the septum is the source of the totipotent cells. There is also an influence on the heart. The enlargement of the stolonic blood vessel has resulted in a reduction of the shorter limb of the heart, so that it opens at one end at the base of the stalk where the stolonic vessel takes origin, fig. 7.

Clavelina, fig. 8, is similar to the distomids in having a hypertrophied stolonic or test vessel and a heart that has been shortened and straightened to open at one end into the stolonic vessel and at the other towards the endostyle. As in *Colella*, the buds arise from the fragmented stolon with the totipotent cells supplied by the septum.

Clavelina and such closely related genera as *Podoclavella* and *Chondrostachys*, alone among the Krikobranchia, might be expected on the basis of size of zooid to possess branchial papillae and internal longitudinal vessels. Since, however, there are indications that the relatively large size is a secondary development, and that these forms may have evolved from the smaller distomids, the absence of such structures may not have much significance. The loss associated with dwarfness might readily be permanent. As in the distomids, the septum of the stolonic vessel is mesenchymatous and arises at the base of the pericardium. This is so in both blastozooid and oozooid of Clavelina, and nowhere does it arise from the epicardium as stated, though not figured, by Van Beneden and Julin (1886) (*cf.* Berrill, 1935, *b*). There is, in fact, no evidence at all that the stolonic septum is any way associated with the epicardium, and the epicardium cannot be said to be an organ primarily concerned with budding, except in certain highly specialized forms.

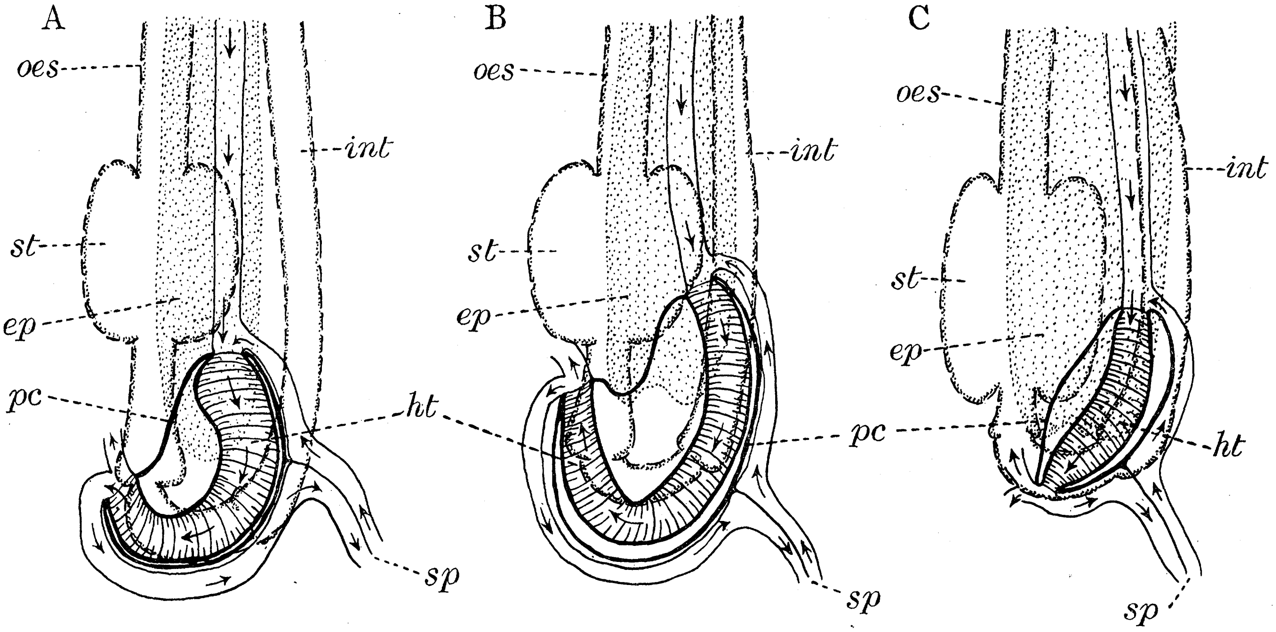

Fig. 6—Reconstructions of the abdominal region based upon whole mounts and transverse sections of A, *Archidistoma aggregata*; B, *Eudistoma olivacea*; and C, *Pycnoclavella aurilucens*. *ep*, epicardium; *ht*, heart; *int*, intestine; *oes*, oesophagus; *pc*, pericardium; *sp*, mesenchymatous septum of vascular stolon; *st*, stomach.

One of such forms is *Diplosoma* (and the family to which it belongs, the Didemnidae). The gut-loop and heart is similar to that of *Distaplia* or *Colella*, although the ventral stolonic vessel has disappeared.

In this genus the epicardia or perivisceral sacs are so small that they of necessity remain separate from one another, and they undoubtedly function primarily as

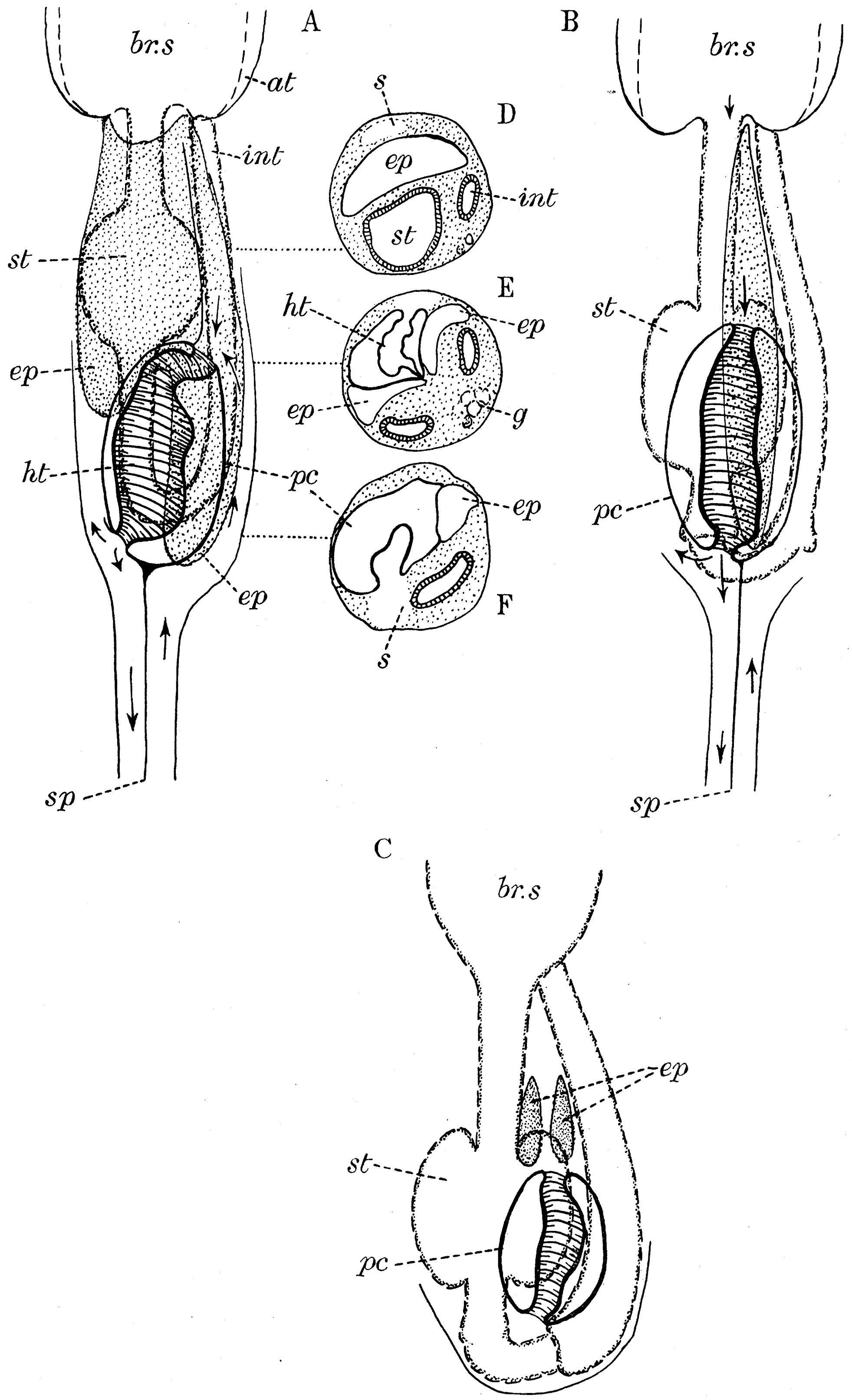

FIG. 7—Reconstructions of A, *Colella thomsoni*; B, *Distaplia rosea*; C, *Diplosoma gelatinosa*; D, E, F, transverse sections of *Colella*. *at*, atrial cavity; *br. s*, branchial sac; *ep*, epicardia; *g*, gonad; *ht*, heart; *int*, intestine; *pc*, pericardium; *s*, blood sinus; *sp*, stolonic septum; *st*, stomach.

organs that give rise to the totipotent cell masses during the complex budding process. Budding is of a type related to that of *Distaplia* (BERRILL, 1935, *b*). The disposition of the various organs is shown in fig. 7C.

In the other forms just mentioned, namely, *Archidistoma*, *Eudistoma*, *Pycnoclavella*, *Distaplia*, *Colella*, and *Clavelina*, the epicardium is an extensive chamber resulting from the fusion of the right and left perivisceral sacs. During development these develop, as in *Ciona*, from posterior diverticula of the pharynx, but in the adults the pharyngeal connexions are lost and the paired condition is denoted only by the pair of anterior horns. The chamber lies always between the gut-loop and the heart, as is indicated by the transverse sections shown in figs. 7 and 8.

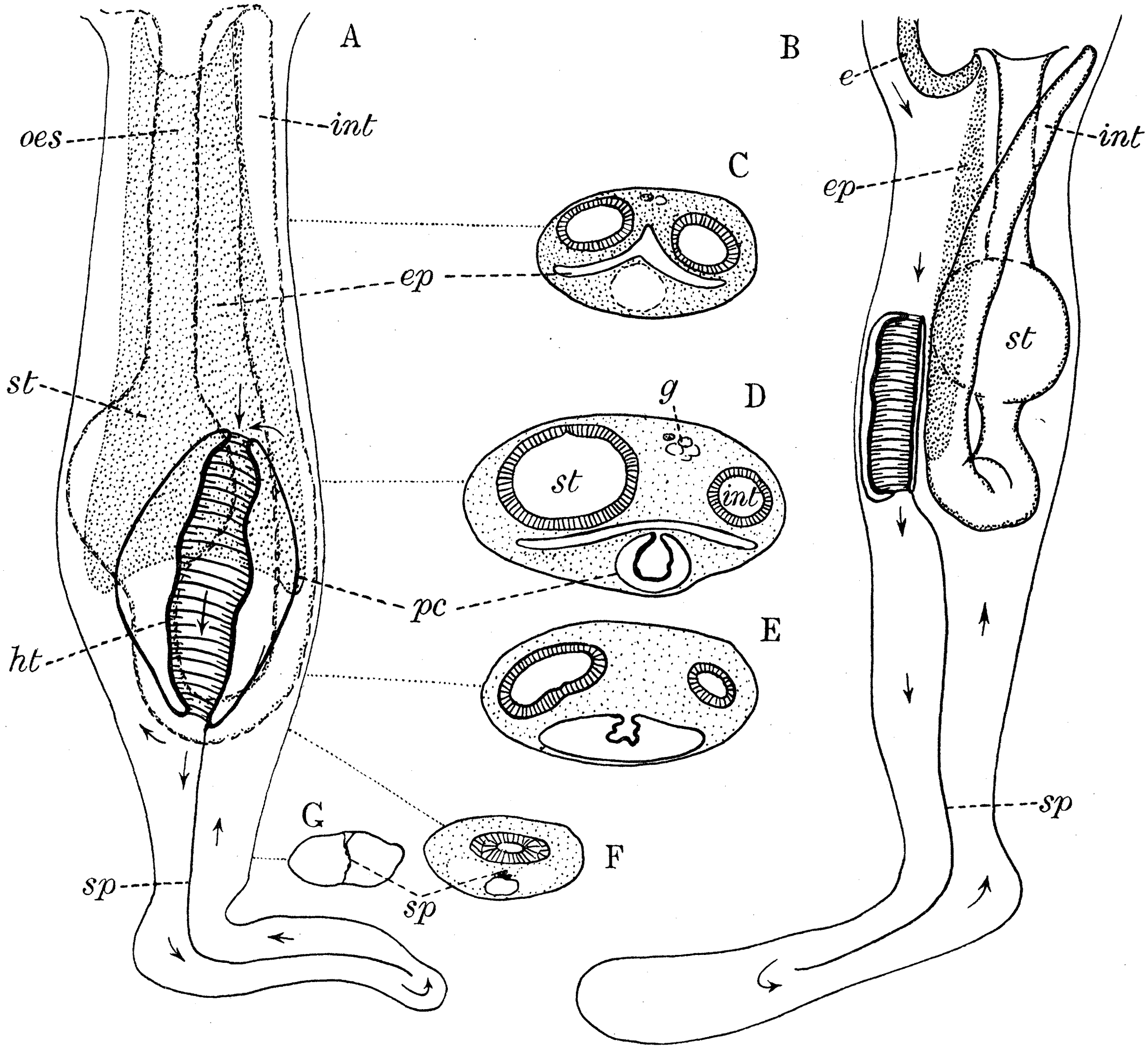

FIG. 8—*Clavelina lepadiformis*. A, reconstruction of adult blastozooid, from ventral side; B, whole mount drawing of young oozoid from side showing relation of stolonic septum to pericardium; C–G, transverse sections of adult blastozooid. *e*, endostyle; *ep*, epicardium; *g*, gonad; *ht*, heart; *int*, intestine; *oes*, oesophagus; *pc*, pericardium; *sp*, stolonic septum; *st*, stomach.

Originating from a diazonid stock, zooids have responded to a dwarfing of the colony not only by a shortening of the body as a whole, but also by a narrowing and extension of the posterior parts, much as in *Tylobranchion.* This has occurred in the family Synoicidae. The stalk elongates, the muscle insertions, the posterior end of the epicardium, and the bend of the heart retain their position relative to the base of the stalk and give the appearance of having descended into the extended stalk, since the gut-loop does not lengthen in a corresponding manner. On the side of the epicardium opposite the heart the gonads are to be found also in the post-abdominal stalk. As there is no posterior formation of a stolonic vessel, there has been no tendency for the U-shaped heart to become straightened out as in the distomids and *Clavelina.*

One genus remains that needs special mention. *Euherdmania* has zooids elongated as in the Synoicidae, but with two important differences : the gut-loop has descended into the extended stalk to a greater distance, while the two epicardial sacs, though losing their openings into the pharynx, remain distinct from one another throughout their length.

The above types are illustrated in fig. 9.

Thus, in one direction the cionid type has evolved through the extension and occupation by the viscera of the fixation stalk. While associated with a reduction in size of colonies among such forms, there has been a dwarfing of the zooid as a whole and in some a hypertrophy of the ventral stolonic vessel, on the one hand, and an extension and narrowing of the body to form a post-abdomen containing all the viscera but the gut-loop on the other.

VI—Ascent of the Viscera

Other ascidians, however, have evolved from the cionid type in a very different direction. In most of these there has been an elaboration of the branchial sac, associated in all probability with increase in absolute body size, since such increase demands a relatively greater increase of respiratory and feeding surfaces. The branchial sac tends to extend posteriorly between the gut-loop and the heart, and in adult forms these two structures become separated from one another and lie on opposite sides of the pharynx. There is, in fact, not only an extension of the branchial sac posteriorly, but also a dislocation or migration forwards of the gut-loop and associated gonads, and the heart and pericardium.

With increase in individual body size and hypertrophy of the branchial sac the fixation stalk dwindles even to a greater extent than it does in *Ciona.* In the Ascidiidae short sub-terminal ampullae apparently represent the stalk shortly after metamorphosis, but not for long. In the families of the Stolidobranchia (Ptychobranchia) the stalk again is recognizable in some species during or shortly after metamorphosis, but either it is without obvious function as in *Botryllus*, *Symplegma*, or *Distomus*, or it forms a creeping stolon-like structure as in certain Molgulae and in *Styela* and *Polycarpa* (Berrill, 1929, 1931). In many species (*Botryllus*, *Symplegma*,

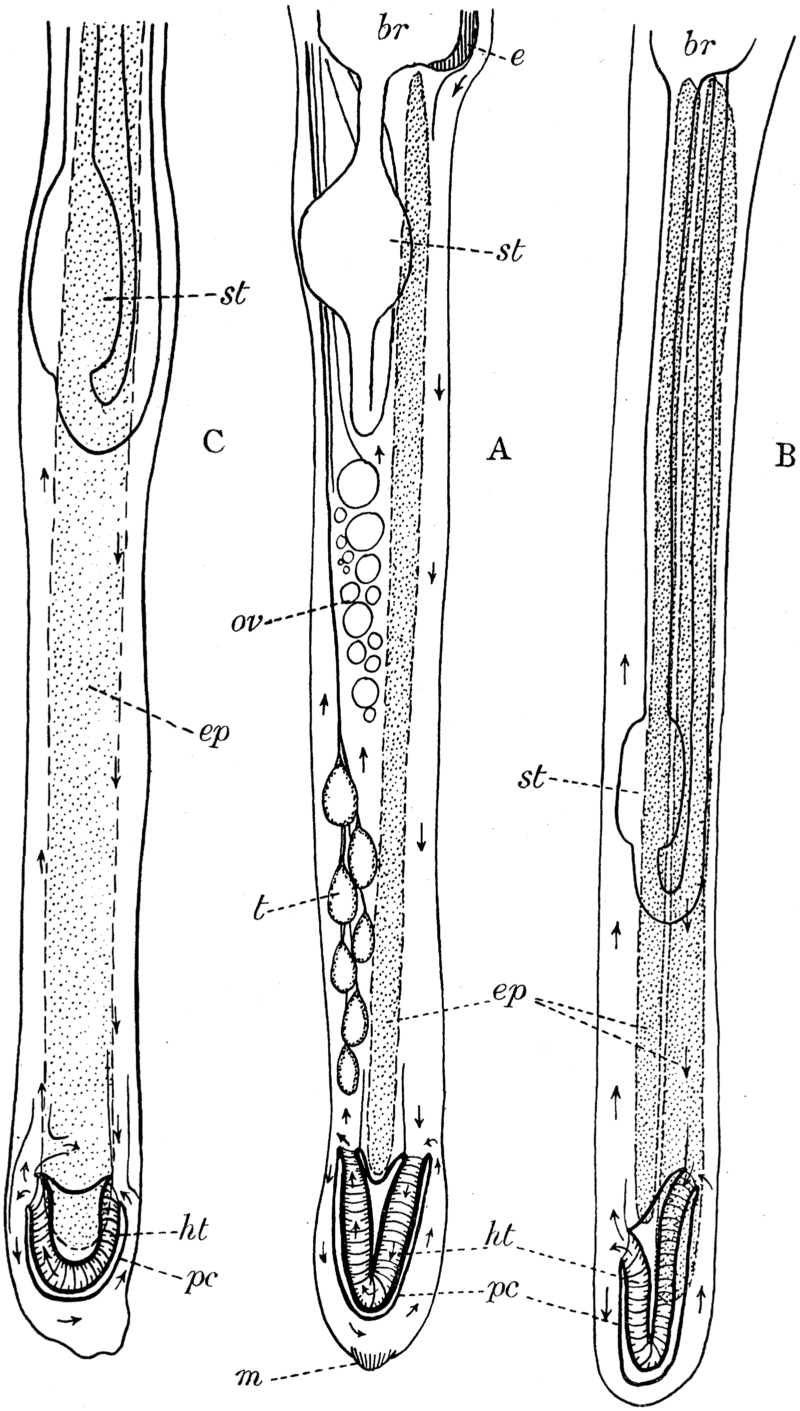

FIG. 9—Reconstructions of abdominal and post-abdominal regions of A, *Sidnyum*; B, *Euherdmania*; and C, *Tylobranchion*, based upon whole mounts and transverse sections. *br*, branchial sac; *e*, endostyle; *ep*, epicardium; *ht*, heart; *m*, posterior insertion of muscle bands; *ov*, ovary; *pc*, pericardium; *st*, stomach; *t*, testes.

Distomus, *Styelopsis*, etc.) the metamorphosing zooid becomes attached by a ring of ectodermal ampullae surrounding the rudiment of the stalk, and the zooid is compressed against the substratum.

In both groups the Ascidiidae and the Stolidobranchia, the stomach and intestinal loop, with its contained gonads, becomes shifted forwards to lie along one side of the branchial sac, while the heart tends to shift forwards along the other side. In spite of such dislocation one end of the heart still opens into the subendostylar vessel, though no longer at the base of the endostyle, and the other end in the region of the stomach.

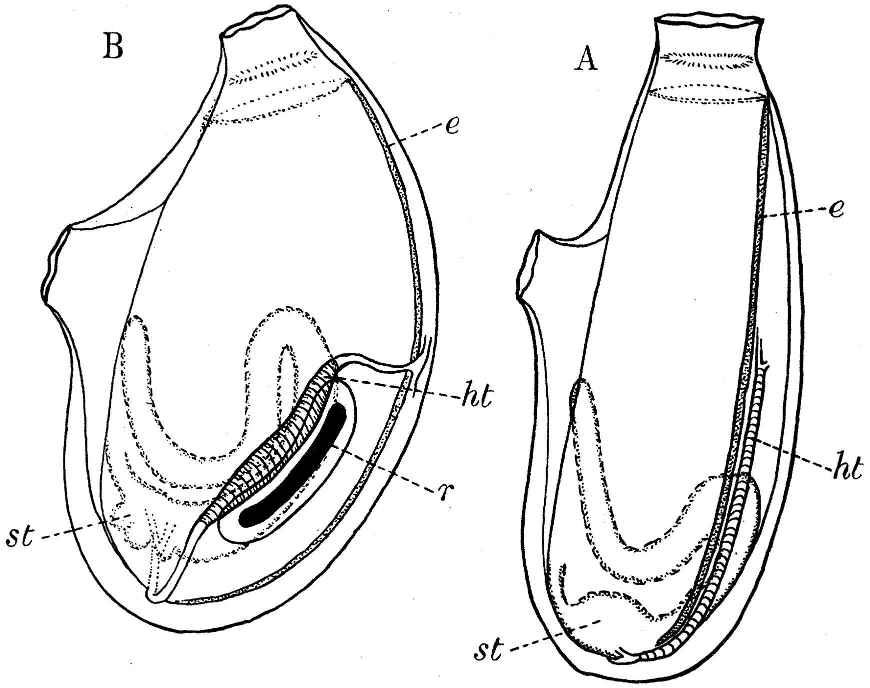

FIG. 10—Adult morphology of *Ascidia* (A) and *Molgula* (B), showing hypertrophy of branchial sac, and translocation of heart and digestive tube, in *Molgula* to opposite sides of sac. *e*, endostyle; *ht*, heart; *r*, renal vesicle; *st*, stomach.

With the shifting of the viscera forwards to the right and left, the perivisceral sacs or epicardia, as they exist in *Ciona* or in *Diazona*, can no longer retain their original relationships. As cavities allowing water to flow over the gut, heart and gonads, their function has to a considerable extent been taken over by the peribranchial cavity or atrial chamber. They do not, however, disappear entirely.

In 1902 DAMAS described the development of the heart in *Molgula* and discovered that during metamorphosis two vesicles are formed from the base of the pharynx. They are in close contact one with another, the one developing to form the heart and pericardium, the other to form the renal vesicle with its enclosed precipitate. The renal vesicle remains in contact with the heart throughout the life of the individual, the two organs growing and elongating together. There is every reason to believe that the renal vesicle is homologous with the epicardium. It is formed from the same region of the pharynx at the same stage of development as the epicardial sacs in *Ciona*. Moreover, the renal function of the epicardia is indicated on other grounds, in the appearance of secretions in those of various forms, and in the establishment of secondary openings into the atrial cavity in *Diazona*. Even in the adult *Molgula* the renal vesicle bears the same relationship to the heart as the epicardium does to that organ in such forms as *Clavelina*, or *Distaplia*.

The renal vesicle remains a single chamber in *Molgula*, and it can be seen in the recently metamorphosed forms of any species of that genus. It can also be seen in the corresponding stage in at least two other genera. As shown in fig. 11, it is discernible also in very young individuals of *Polycarpa* of the family Styelidae, and of *Ascidiella* of the family Ascidiidae, and in *Ascidiella* at least it develops as in *Molgula*, from the base of the pharynx.

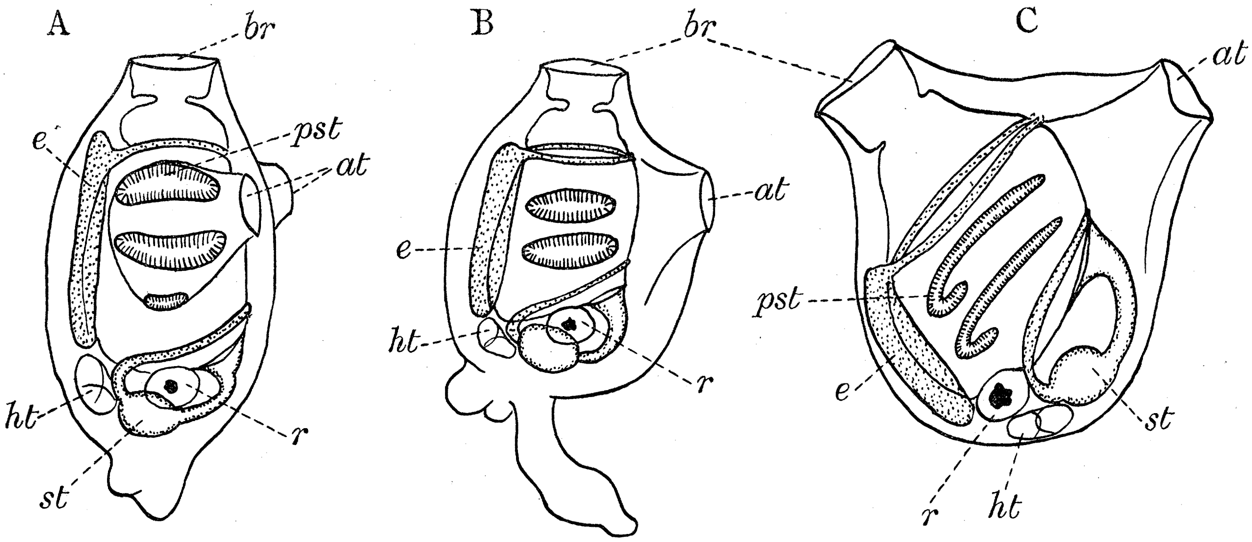

FIG. 11—Newly-functional oozooids of A, *Ascidiella aspersa*; B, *Polycarpa rustica*; and C, *Molgula citrina*. *at*, atrial siphon (*s*) ; *br*, branchial siphon ; *e*, endostyle ; *ht*, heart ; *pst*, protostigma ; *r*, renal vesicle (epicardium) ; *st*, stomach.

Thus the Ascidiidae and Stolidobranchia are united together by the dislocation of the viscera and the transformation of the perivisceral sacs or epicardia into a renal vesicle. In this last respect there is more uniformity among the recently metamorphosed individuals than among their respective adults. Only in *Molgula* does the renal vesicle remain single and retain its primitive relationship to the heart. In the Ascidiidae and *Corella* the vesicle, instead of growing as a single organ, multiplies or subdivides until the gut wall and region between gut and heart becomes congested with innumerable small renal vesicles.

In the Styelidae, Botryllidae and some Pyuridae, the vesicle or vesicles are not recognizable in the adult, although it is discernible in *Microcosmus*, a genus linking the Pyuridae with the Molgulidae.

In other respects the Stolidobranchia and the Ascidiidae differ considerably. The Ascidiidae are the more primitive in that they form a pair of lateral peribranchial chambers in development that later fuse to form the median atrium. In the Stolidobranchia the atrial aperture is median and single from the very beginning. The gonads in the Ascidiidae are to be found only within the loop of the gut, while in the Stolidobranchia bilaterality is more pronounced and gonads are formed on the opposite side as well.

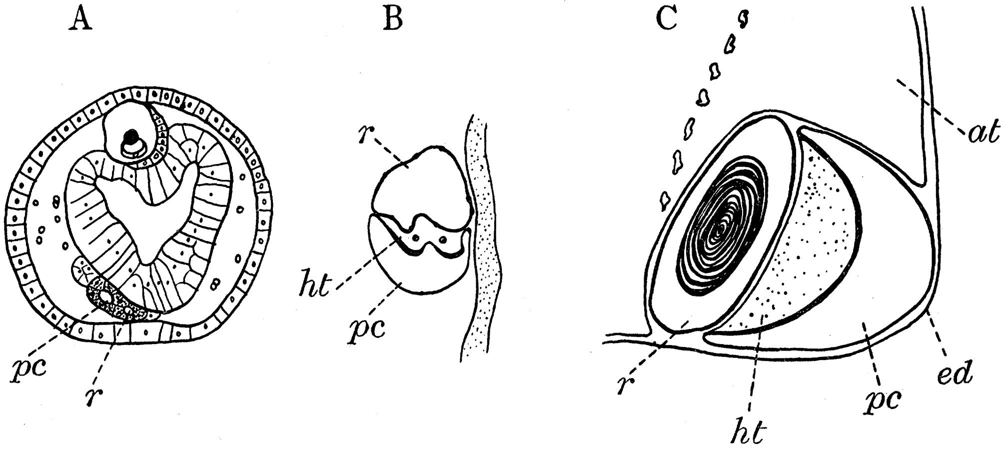

FIG. 12—Relationship between renal vesicle and heart in *Molgula*. A, origin of pericardium and renal vesicle from base of pharynx in tadpole of *Molgula echinosiphonica* (after DAMAS); B, renal vesicle, heart, and pericardium in *Molgula* similar to stage shown in fig. 11 C (after DAMAS); C, condition in adult *Molgula*. *at*, atrial cavity; *ed*, epidermis; *ht*, heart; *pc*, pericardium; *r*, renal vesicle.

The evolution from a cionid type to form the above-mentioned groups may thus be visualized as occurring in two stages. In the first, as represented by the Ascidiidae, there is a translocation of the gut-loop and gonads along one side and the heart towards the other side of the branchial sac, while the epicardia are transformed into the renal vesicle. In the second there is intensification of bilaterality as evidenced by gonads on each side, a developmental abbreviation in the formation of the atrial siphon, and an elaboration of the branchial sac so that its internal surface is increased by a series of pleats or folds. In the Styelidae (inclusive of Botryllidae) lateral or atrial budding has been acquired.

VII—THE PEROPHORIDAE

There remains to be discussed the family Perophoridae. In many respects it would seem to be but an early step in the change from the cionid to the ascidiid type, but there are indications that this is not so.

In common with the Ascidiidae the intestine has been shifted forwards along the left side of the branchial sac, together with the gonads, although the stomach lies posteriorly to the sac as in *Ciona*. In the larger genera such as *Ecteinascidia*, internal longitudinal vessels are to be found in the branchial wall, as in *Ascidia*, *Ciona*, and *Diazona*. In the smaller *Perophora* the papillae that give rise to the vessels in larger forms are present.

If the Perophoridae do not represent a step in the trend from *Ciona* to the Ascidiidae and Stolidobranchia, then they must have evolved from the stock of those forms in which the viscera have descended the stalk. For this there is some evidence. There are two valves between the posterior end of the stomach and the hind part of the intestine that suggest forcibly that the gut-loop once occupied the stalk as in *Distaplia* (*cf.* *Ecteinascidia* and *Distaplia*, etc., in figs. 7 and 13). The oviduct is short and wide and barely reaches the base of the atrial chamber, a condition typical only of the smaller types with descended viscera. There is a hypertrophied stolonic vessel with a mesenchymatous septum. Buds develop from the stolon as in *Clavelina* and *Colella*, although without fragmentation or isolation from the parent zooid. Lastly, there is no trace of the epicardium either in its original form or as a renal vesicle at any stage of development.

Since the ventral test vessel with its septum is present in *Ciona*, its hypertrophy and development as a budding stolon in Perophoridae could have occurred without the descent of the viscera into the stalk.

Altogether the evidence is conflicting, and it is quite possible that the Perophoridae may represent an evolution from the primitive cionid stock independently of the two major trends of descending and ascending viscera described above.

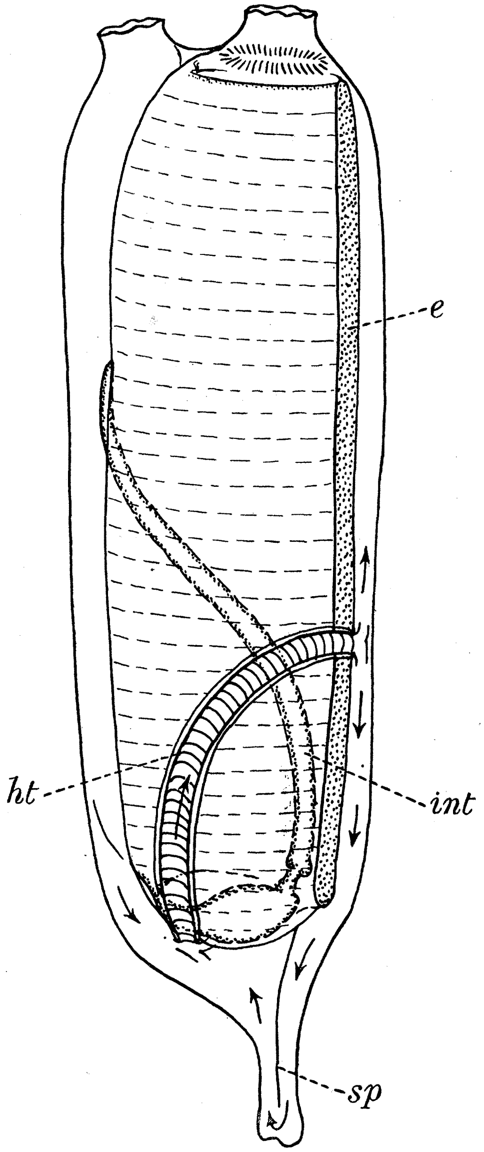

Fig. 13—Adult morphology of *Ecteinascidia turbinata*, showing translocation of heart and intestine. *e*, endostyle; *ht*, heart; *int*, intestine; *sp*, mesenchymatous septum.

The Perophoridae illustrate again the relation between body size and branchial structure, and the danger of placing great emphasis on the absence of longitudinal vessels. *Perophora* and *Ecteinascidia* are very closely related, yet the dwarfing that is evident in the former has resulted in the non-development of those vessels. Similarly, while branchial folds are the main characteristic of the order Stolidobranchia, they are absent in the dwarfed botryllids, *Symplegma* and in *Eugyra*.

It is considered, moreover, that while the Stolidobranchia (Ptychobranchia) form a natural order or suborder, the Phlebobranchia (Dictyobranchia) are very heterogeneous and that certain members of this order are more closely related to members of the Aplousobranchia (Krikobranchia) than to other members of their own order.

It is proposed, therefore, to construct a classification based more upon the organization as a whole, and especially upon structure less likely to have undergone convergent evolution and less dependent upon mere body size than that of the branchial sac.

VIII—Classification

The various regions, organs or structures of ascidians upon which classification may be based are principally the branchial sac, the post-branchial intestine, the gonads, the heart and the epicardium. Of these, objections have already been raised against the first. The relative position and the appearance of the post-branchial intestine or gut-loop is not entirely satisfactory as a basis, and in any case does not present sufficient variability. The same applies to the gonads, since they seem to have certain positional relationships to the intestine, and also are influenced structurally to some extent by the size of the individual. The heart again exhibits insufficient variation, and, while all the above-mentioned structures are of great diagnostic value when taken together, the epicardium alone as a single organ seems to reflect the major divisions of the class.

The epicardium will accordingly be used as the basis for separating the class into its orders, the other structures for dividing those orders into families and subfamilies. Inasmuch as the epicardia, in their development and general nature, seem more than reminiscent of the vertebrate coelom, the order names will be based upon this similarity.

Whether or not *Ciona* be as primitive as suggested earlier, there is no doubt that it stands apart from other ascidians, and inasmuch as the epicardia are believed to exist in this genus in their most primitive form, it is suggested that the genus comprises in itself the order Diplocoela.

In contrast to the unique structure of *Ciona*, there are innumerable forms in which the epicardia have descended the stalk with the gut-loop and have lost the openings into the pharynx. With the exceptions of *Diplosoma* and *Euherdmania* the two chambers have fused, and these two exceptions are by no means necessarily primitive retentions. This large group is designated as the order Epicardiocoela.

In the Perophoridae the chambers have apparently been lost entirely and the family in many ways stands apart from other ascidian types. In consequence it is felt justifiable to construct a separate order for its reception, namely, the Acoela.

The remaining ascidians, the Ascidiidae, Rhodosomatidae, and the families of the order Stolidobranchia (Ptychobranchia) comprise a natural group so far as the epicardia form, at some stage of development, a closed vesicle containing a renal concretion. The excretory function of the epicardia is thus emphasized, and it is proposed to include all these families within the order NEPHROCOELA.

Of the above orders, the Diplocoela and the Acoela are so small that no subdivision of the order and single family in either is necessary or desirable. In the Epicardiocoela and Nephrocoela the need is great.

The Epicardiocoela contain the old families Diazonidae, Synoicidae, Didemnidae and Clavelinidae (including Distomidae). Of these the first three are fairly well defined, but the last is decidedly heterogeneous. It is considered that the distomids, *Archidistoma* and *Eudistoma* are virtually dwarfed diazonid types, and the family Diazonidae will be re-defined to include these genera and to exclude *Tylobranchion*, a genus resembling *Diazona* rather than the Synoicidae only in the presence of branchial papillae. The Synoicidae will be enlarged to include all Epicardiocoela with post-abdominal extensions containing heart, epicardia, gonads, and muscle, namely, the Synoicidae plus *Tylobranchion* and *Euherdmania*, although these may represent three lines of parallel evolution from a diazonid stock. In view of this possibility and of certain distinguishing features between the three groups, they will be defined as subfamilies. It is of interest that there is a striking resemblance between the tadpole larvae of *Eudistoma* and those of the Synoicinae, suggesting the origin of the last group from the former.

The genera *Clavelina*, *Chondrostachys*, *Sigillina*, *Podoclavella*, *Colella*, and *Distaplia* are all characterized by a hypertrophy of the ventral stolonic vessel and by a correlated straightening-out of the originally V-shaped heart. *Pycnoclavella* in some ways seems to be intermediate in type between the above and the *Eudistomid*, although it is also highly specialized. For convenience it is included with the above as part of the family Clavelinidae. This family remains somewhat heterogeneous, and, on the basis of mode of budding, could be divided into four sub-families.

The remaining family of this order, the Didemnidae, has no need of modification. The nature of the heart, general organization, and method of budding suggest a relationship with *Distaplia*, the enlarged ventral stolon having been lost entirely.

Thus the Didemnidae and Clavelinidae are connected by the nature of the heart, and so are the Diazonidae and Synoicidae. To indicate these relationships, the Epicardiocoela is divided into two sub-orders, the Dicardia containing the two last-mentioned and the Unicardia the first two families.

The Nephrocoela must also be divided into two sub-orders, one to include the families Ascidiidae and Rhodosomatidae, the other the Styelidae, Botryllidae, Pyuridae, and Molgulidae. In current classifications these two groups are separated by virtue of differences in the branchial sac, the latter alone having branchial folds. Since, however, dwarfed types of this last are without folds, it is preferred to base the separation upon other characters, and with this object names are revived from Perriers's classification, Enterogona and Paragona. In the sub-order Enterogona the gonads occur only on one side of the body and within the primary loop of the gut. In the Paragona they occur on each side of the body and with no fixed relation to the primary gut-loop on that side. Reasons for the subordination of the Botryllidae within the Styelidae have been given elsewhere (Berrill, 1932).

The full classification as indicated above, together with brief definitions of the various groups, is given below.*

Class Ascidiacea

Order	Sub-Order	Family	Sub-Family	Genera
1. Diplocoela (Epicardia in form of two perivisceral sacs opening anteriorly into lumen of pharynx.)		*Cionidae*		*Ciona*
2. Epicardiocoela (Epicardia descended into stalk with rest of viscera, openings to pharynx lost, and the two chambers usually fused.)				
	A. Dicardia (Heart V-shaped with raphe not closed by epicardium.)			
		Diazonidae (No post-abdomen, budding by abdominal constriction.)		*Rhopalea* *Diazona* *Archidistoma* *Eudistoma*
		Synoicidae (Post-abdomen, budding by post-abdominal constriction.)		
			Synoicinae	*Morchellium* *Sidnyum* *Polyclinum* *Amaroucium* *Aplidium*
			Euherdmaniae	*Euherdmania*
			Tylobranchionae	*Tylobranchion*

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* The classification given here is to be regarded as tentative and not in its final form.

CLASS ASCIDIACEA—(continued)

Order	Sub-Order	Family	Sub-Family	Genera
	B. UNICARDIA (Heart straight with raphe closed by epicardium.)			
		Clavelinidae (Ventral stolonic vessel enlarged.)		*Archiascidia* *Clavelina* *Podoclavella* *Chondrostachys* *Sigillina* *Colella* *Distaplia* *Pycnoclavella*
		Didemnidae (Ventral stolonic vessel absent.)		*Didemnum* *Diplosoma* *Coelocormus*
3. ACOELA (Without trace of epicardia.)		*Perophoridae*		*Perophora* *Perophoropsis* *Ecteinascidia*
4. NEPHROCOELA (Epicardia in form of renal vesicle or vesicles.)				
	A. ENTEROGONA (Gonads on one side of body only.)			
		Ascidiidae		*Ascidia* *Ascidiella*
		Rhodosomatidae		*Rhodosoma* *Chelyosoma* *Corella*
	B. PARAGONA (Gonads on each side of body.)			
		Styelidae		*Styela* *Polycarpa* *Styelopsis* *Polyandrocarpa* *Distomus* *Alleocarpa* *Stolonica* *Symplegma* *Botryllus* *Botrylloides*

Class Ascidiacea—(continued)

Order	Sub-Order	Family	Sub-Family	Genera
		Pyuridae		*Pyura*
				Tethyum
				Boltenia
				Culeolus
				Microcosmus
				Forbesella
		Molgulidae		*Molgula*
				Eugyra

A Comparison of Current and Proposed Classifications

Classification of Lahille and of Seeliger		Proposed Classification	
Order	Family	Sub-Order	Order
Stolidobranchia or Ptychobranchia	Molgulidae	Paragona	Nephrocoela
	Pyuridae		
	Styelidae		
	Botryllidae		
Phlebobranchia or Dictyobranchia	Ascidiidae	Enterogona	
	Rhodosomatidae		
	Perophoridae		Acoela
	Cionidae		Diplocoela
	Diazonidae	Dicardia	Epicardiocoela
Aplousobranchia or Krikobranchia	Synoicidae		
	Distomidae		
	Didemnidae	Unicardia	
	Clavelinidae		

While the above classification is based primarily upon the nature of the epicardia, and partly also on the heart, a grouping of the ascidians according to their mode of budding (*cf.* Berrill, 1935, *b*) would approximate closely to the same scheme. The difference between that proposed and the current classifications is that the order Phlebobranchia (Dictyobranchia) is regarded as most heterogeneous and has been split so that two families, the Cionidae and Perophoridae, become two orders, and the remaining families are placed among the other two orders, the Stolidobranchia and Aplousobranchia, the enlargement of which necessitates re-definition.

It has been stated already that since there is a correlation between the body size of an individual and the presence or absence of branchial papillae, and also between size and the fusion of papillae to form inner longitudinal vessels, that such differences are of questionable value when used for the distinction of orders, as in distinction between the Phlebobranchia (Dictyobranchia) and the Aplousobranchia (Krikobranchia). This objection is supported by the fact that distinction itself is not exact, for in the Synoicid genera Polyclinum and Glossophorum, both with relatively large branchial sacs, papillae are definitely present.

Apart from this relegation of branchial structure to a place of secondary importance, the classificatory scheme proposed agrees with the more general conception of ascidian relationships.

In fig. 14 is summarized diagrammatically the interrelationships of the various orders, families and genera.

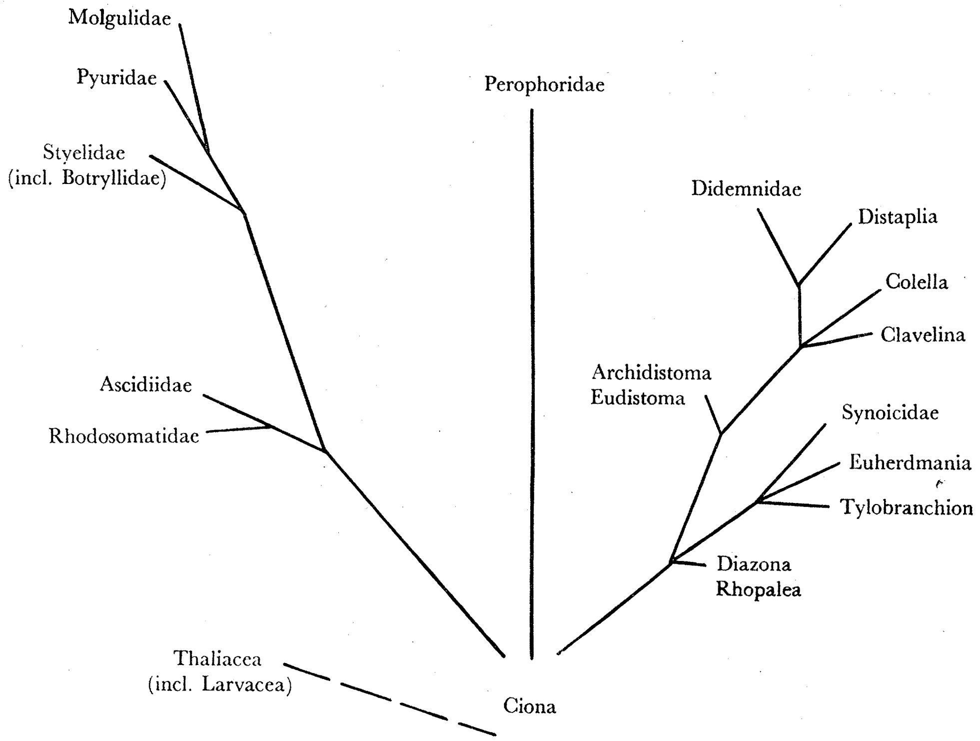

FIG. 14—Diagram of probable interrelationships of ascidian families.

IX—THE THALIACEA AND LARVACEA

If the foregoing classification reflects in any way the evolutionary trends within the Ascidiacea, it should be possible to relate to them in some way the evolution or origin of the Thaliacea (*Pyrosoma*, *Doliolum* and *Salpa*) and Larvacea. In a recent paper GARSTANG (1928) has assembled evidence to show that the Larvacea, far from being surviving primitive ancestral forms as commonly supposed, are in reality highly specialized neotenous doliolids. With this contention the writer is in full agreement, and the development of Oikopleura recorded by DELSMAN (1912), though not considered by GARSTANG, is further indication of an extreme specialization rather than a primitive nature of these forms.

The Thaliacea as a whole evidently have originated from a single stock, as evidenced by their peculiar mode of budding from an epicardial outgrowth at the base of the endostyle. In all three thaliacean families the branchial sac, atrium and musculature are highly specialized, and are obviously related to the demands of a free-swimming pelagic life.

The gonads are single and lie close to the loop of the intestine, and this fact, in the absence of positive evidence to the contrary, precludes relationship with the *Paragona* (Stolidobranchia). The presence of epicardia excludes close affinity with the Acoela (Perophoridae), in spite of some resemblance in the methods of budding. At the same time, no structure in the Thaliacea suggests that the viscera have ever descended a fixation stalk as in the Epicardiocoela (Diazonidae plus Aplousobranchia), and the position of the post-branchial gut and the heart resembles that in newly metamorphosed individuals of *Ciona*, *Diazona*, or *Ascidia*. This relative position of gut and heart to the rest of the body is, in fact, extremely primitive. In young forms the epicardia retain their openings with the pharynx, although the distal epicardial tissue is specialized for budding and does not form the lining of the perivisceral cavity as in *Ciona*.

Thus, of the various types of ascidians, the Thaliacea can be related only with *Ciona*, and the evidence even in this case is merely negative, suggesting that the Thaliacea may have evolved at almost any stage during the evolution of the stock that culminated in *Ciona*.

X—FUNCTION OF THE EPICARDIUM

The epicardium obviously has a variable function among ascidians. In *Diplosoma* and in the Thaliacea it is without doubt concerned primarily with the formation of totipotent strands in connexion with regeneration and budding. In the Molgulidae and some other families it is equally certain that the epicardium has no concern with budding and is primarily an organ of excretion. It would seem likely, therefore, that the two functions just described are two extreme specializations of the epicardium and that primitively both functions might be present in a less spectacular form. Secretions into the lumen in the Epicardiocoela suggest an excretory function, especially as the epicardium is always in close association with the pericardium even in the Molgulidae, while the secondary apertures into the atrial cavity in *Diazona* reinforces this suggestion. At the same time, in many of these forms the epicardial lining epithelium is the source of all new cells during regeneration or budding. This, however, merely denotes the absence of any significant or irreversible specialization, for totipotency is characteristic of other tissues that have normally a comparatively passive function. Thus the cells of the mesenchymatous septum of the vascular stolon are also totipotent, although the septum has no developmental or other connexion with the epicardium, as has been commonly believed. It arises from the base of the pericardium even in the oozooid of *Clavelina*, the one case where it has been stated to be of epicardial origin, and it seems to be merely a septum of mesenchyme induced by vascular exigencies. Other totipotent cells form the lining of the atrial cavity, as is shown by budding in the Styelidae.

Thus from the point of view of function, the epicardium seems to be most primitive again in *Ciona*. There it allows water drawn into the branchial chamber to circulate slowly around the heart, intestine and gonads, and yet in the rare occurrence of the loss of anterior tissues in this genus, the new formative cells arise from the epicardial lining. There is therefore some suggestion of excretory function of the epicardium in *Ciona* and definite evidence of an unspecialized condition of the cells forming it. In many ways, in fact, the ascidian epicardium as it appears in *Ciona* resembles the coelom of the higher chordates. This question will be considered more fully elsewhere.

XI—Summary

An account is given of the adult morphology of the heart and epicardium in the following genera: *Ciona*, *Diazona*, *Rhopalea*, *Tylobranchion*, *Euherdmania*, *Sidnyum*, *Eudistoma*, *Archidistoma*, *Distaplia*, *Colella*, *Diplosoma*, *Pycnoclavella*, *Clavelina*, *Perophora*, *Ecteinascidia*, *Ascidia*, and *Molgula*.

The development of the heart and epicardium is described for *Ciona*, *Diazona*, *Ascidia*, *Polycarpa*, and *Molgula* (*i.e.*, genera all with small eggs).

The heart develops as an infolding of the pericardium, which in turn is an evagination from the base of the pharynx. At first it is a straight tube opening at one end in the region of the stomach, at the other into the subendostylar vessel at the base of the endostyle. With growth of the individual in *Ciona* the heart becomes V-shaped as the result of extension in length between two relatively fixed points. In *Diazona*, and in the synoicids and distomids, it sinks into the stalk together with the rest of the viscera, the bend of the heart resting in the base of the stalk. In the clavelinids and certain distomids hypertrophy of the ventral or posterior stolonic vessel has had the effect of shortening and straightening the heart, so that the end which primitively opened near the stomach now opens posteriorly. In the Perophoridae the heart has become dissociated from the rest of the viscera and extends as a long tube along one side of the branchial sac, the end that opens into the subendostylar vessel having become shifted anteriorly. In the Ascidiidae, Styelidae, Pyuridae, and Molgulidae, a similar shifting has occurred, although the relative displacement of the heart along one side of the branchial sac is less striking than the shifting of the intestinal loop forwards along the other side.

The epicardium in *Ciona* and *Diazona* develops as a pair of evaginations from the posterior end of the pharynx. In *Ciona* these grow and envelop the viscera, maintaining open communication. In *Diazona* the openings are lost, the two sacs fuse to form a single chamber between the heart and intestinal loop, and descends with the viscera as a whole into the stalk, similar to the conditions in the Synoicidae, Didemnidae, Distomidae, and Clavelinidae. In the Perophoridae there is no sign of the epicardium in the adult or at any stage of development.

In *Ascidia*, *Polycarpa*, and species of *Molgula* it develops as a median evagination from the posterior end of the pharynx, becomes a closed vesicle, and contains a renal concretion. Such vesicles become numerous in adult *Ascidia*, disappear in *Polycarpa*, but remain recognizable and single in Molgulids and the pyurid *Microcosmus*.

Evidence is cited to show the doubtful value of branchial structure as a basis for classification, and the relationship between such structure and the absolute size of an ascidian.

A new classification of the class Ascidiacea is proposed, based primarily upon the nature of the epicardium. The result has been mainly to break up the heterogeneous order Phlebobranchiata (Dictyobranchiata), and to form five orders in place of three. It is believed that the classification proposed is a more probable reflexion of the course of evolution within the Ascidiacea than those displaced.

It is suggested that the function of the epicardium is to be found in its most primitive condition in *Ciona*, where it is of a coelomic nature, partly excretory, but so unspecialized that its cells can play a formative part in regeneration of missing parts. It is believed that the importance of the epicardium in the budding processes of such forms as *Diplosoma*, *Aplidium*, and the Thaliacea, and as an excretory organ in the Ascidiidae, Styelidae, Pyuridae, and Molgulidae is the result of extreme specialization in different directions of functions present, but poorly developed, in *Ciona*.

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